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ANAEROBIC-AEROBIC TREATMENT PROCESS FOR THE REMOVAL OF PRIORITY POLLUTANTS

by

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16. ABSTRACT

The removal of 4,6-dinitro-o-cresol (DNOC) was investigated using an anaerobic recycle fluidized bed reactor as a pretreatment stage followed by an activated sludge reactor.

The DNOC was completely converted during the anaerobic pretreatment stage for influent DNOC concentrations as high as 600 mg/l. While complete conversion of DNOC occurred during the anaerobic pretreatment stage, there was only 25% COD removal. The subsequent aerobic activated sludge stage reduced the anaerobic stage effluent COD by 80%, resulting in about 85% overall removal.

Batch tests established a range of DNOC loading rates for the anaerobic fluidized bed. The batch tests also indicated that DNOC did not degrade in the absence of a readily biodegradable co-substrate, and could not be used as a single carbon source by the anaerobic bacteria. This investigation used sucrose as the co-substrate. Anaerobic DNOC biodegradation was found to be a function of sucrose concentration.

Previous investigation of aerobic treatment of DNOC using conventional activated sludge process showed that DNOC removal is less than 25% and the concentration of DNOC that is tolerated by activated sludge microorganisms is only about 50-60 mg/l. The present investigation demonstrated that anaerobic-aerobic treatment is an effective treatment process for the removal of DNOC.

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FOREWORD

EPA is charged by Congress to protect the Nation's land, air, and water systems. Under a mandate of national environmental laws focused on air and water quality, solid waste management and the control of toxic substances, pesticides, noise, and radiation, the Agency strives to formulate and implement actions which lead to a compatible balance between human activities and the ability of natural systems to support and nurture life.

The Robert S. Kerr Environmental Research Laboratory is the Agency's center of expertise for investigation of the soil and subsurface environment. Personnel at the Laboratory are responsible for management of research programs to: (a) determine the fate, transport and transformation rates of pollutants in the soil, the unsaturated zone and the saturated zones of the subsurface environment; (b) define the processes to be used in characterizing the soil and subsurface environment as a receptor of pollutants; (c) develop techniques for predicting the effect of pollutants on ground water, soil and indigenous organisms; and (d) define and demonstrate the applicability and limitations of using natural processes, indigenous to the soil and subsurface environment, for the protection of this resource.

This project was undertaken to evaluate the use of anaerobic pretreatment for combined industrial/municipal waste treatment systems that treat organic priority pollutants with an aerobic activated sludge process. The results of this study indicate that 4,6-dinitro-o-cresol, a priority pollutant, can be almost completely removed by anaerobic pretreatment provided a readily biodegradable co-substrate is present. This information should be useful to those responsible for regulating, designing, and operating combined industrial/municipal waste treatment systems.

Clinton W. Hall

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ABSTRACT

The removal of 4,6-dinitro-o-cresol (DNOC), a phenolic priority pollutant, and the removal kinetics were investigated using an anaerobic recycle fluidized bed reactor as a pretreatment stage followed by activated sludge reactor as the aerobic treatment stage.

The DNOC was completely converted during the anaerobic pretreatment stage (anaerobic bed effluent DNOC concentration <<1 mg/1), when the influent DNOC concentrations were as high as 600 mg/1. It was also found that while 100% conversion of DNOC occurred during the anaerobic pretreatment stage, there was approximately 25% COD removal. The subsequent aerobic activated sludge stage reduced the anaerobic stage effluent COD by approximately 80%, resulting in about 85% overall removal.

Batch test studies employing controlled feed to the anaerobic microorganisms provided preliminary data that were used for establishing a range of DNOC loading rate onto the anaerobic fluidized bed. The batch test studies also provided early indications that DNOC was highly toxic to the anaerobic microorganisms in the absence of a readily biodegradable co-substrate, and could not, therefore, be used as a sole carbon source by the anaerobic bacteria. Sucrose was used as the co-substrate in this investigation. The anaerobic DNOC biodegradation was found to be a function of sucrose concentration.

Previous investigation of aerobic treatment of DNOC using conventional activated sludge process showed that DNOC removal is less than 25% and the concentration of DNOC that is tolerated by activated sludge microorganisms is only about 50-60 mg/1. The results of the present investigation demonstrate that the anaerobic-aerobic treatment process is an effective treatment process for the removal of DNOC.

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ABBREVIATIONS AND SYMBOLS

ABBREVIATIONS

AC -- anaerobic column
AS -- activated sludge
ATP -- adenosine triphosphate
COD -- chemical oxygen demand
COD_s -- soluble chemical oxygen demand
DNOC -- 4,6-dinitro-o-cresol
EC_{50} -- effective concentration of toxicant causing 50% reduction in light output in Microtox^{TM} toxicity test
HRT -- hydraulic retention time (based on influent flow rate)
μmhos/cm -- micromhos per centimeter
mV -- millivolts
N.D. -- not detected
ORP -- oxidation-reduction potential
Q -- flow rate
RHRT -- recycle hydraulic retention time
SS -- suspended solids
t -- time
TOA -- total organic acids
TOC -- total organic carbon (soluble)
VSS -- volatile suspended solids

SYMBOLS

CH_4 -- methane
CO_2 -- carbon dioxide
HCO_3^- -- bicarbonate ion
$NaHCO_3$ -- sodium bicarbonate
Na_2SO_4-H_2SO_4 -- sodium sulfate-sulfuric acid solution
N_2 -- nitrogen
$(NH_4)_2HPO_4$ -- dibasic ammonium phosphate
NO_2^- -- nitrite ion

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SECTION 1

INTRODUCTION

4,6-Dinitro-o-cresol (DNOC), one of the U.S. EPA priority pollutants, is a yellow crystalline solid derived from ortho-cresol. DNOC was introduced in 1892, in its potassium salt form, as the active ingredient of the pesticide "Antinonin", used for controlling the nun moth. It is among the most toxic of all compounds used as herbicides. Currently, it is used primarily as a blossom-thinning agent on fruit trees and as a fungicide, insecticide, and miticide on fruit trees during the dormant season. It also has limited use in the dye stuff industry and for other minor industrial purposes.

DNOC has been detected in the following industrial wastewaters: organics and plastics manufacturing industries, pesticides manufacturing industry, rubber manufacturing industry, wood-preservatives industry, pharmaceutical manufacturing industry, coal mining, foundries, and iron and steel manufacturing industry (5,6).

Literature on the degradation of DNOC shows that it undergoes biodegradation by pure soil microorganisms cultures under aerobic conditions. However, results from laboratory scale activated sludge studies (1) show that the removal of DNOC in the activated sludge process is less than 25% and the concentration of DNOC that is tolerated by activated sludge microorganisms is only about 50-60 mg/1. These results indicated that the biological treatment of DNOC by activated sludge process is unsatisfactory. Data from physical and chemical treatment of DNOC (5,67,52) also show that these treatment alternatives might be technologically or economically unfeasible. Very little information is available about the anaerobic degradation of most organic compounds, but some data indicate that aromatic nitro groups can be reduced to amino groups in anaerobic conditions. It was also shown that some nitro-aromatic compounds that can be degraded under anaerobic conditions are not degraded under aerobic conditions. All these facts encouraged the study on the fate of DNOC under anaerobic conditions.

Treatment of DNOC by single anaerobic process alone risks inactivating the environment-sensitive methanogens, in which case the organic loading of the pollutant will not be reduced. It is also known that the benzene nucleus, which most likely may still exist if DNOC is just cometabolized to another aromatic compound during the anaerobic process, will undergo ring cleavage in the presence of oxygenase under aerobic conditions. Anaerobic pretreatment-aerobic treatment scheme for treating wastes containing DNOC is, thus, very promising in achieving the purposes of biodegrading the priority pollutant, DNOC, as well as lowering the organic loading of the waste.

The research reported here describes a pilot scale study on the anaerobic-aerobic biological degradation of 4,6-dinitro-o-cresol. It provides an assessment of the parameters controlling its biodegradation, and an assessment of its biodegradation by-products and their toxicity.

SECTION 2

CONCLUSIONS

A biological treatment system for the removal of 4,6-dinitro-o-cresol (DNOC), a phenolic priority pollutant, has been designed and tested. The system was comprised of an anaerobic fluidized bed reactor as a pretreatment stage followed by an activated sludge reactor as the aerobic treatment stage. The results of this investigation show that, in contrast to the failure of the conventional activated sludge process to remove DNOC (removal of DNOC in the activated sludge process is less than 25% and the inhibitory threshold of DNOC by the activated sludge microorganism is 50-60 mg/l), DNOC was completely converted during the anaerobic pretreatment stage. The anaerobic bed effluent DNOC concentration was less than 1 mg/l, with a DNOC concentration in the influent up to 600 mg/l. It was also found that, while 100% conversion of DNOC occurred during the anaerobic pretreatment stage, there was less than 25% removal of COD during that stage. In the activated sludge process most of the COD was removed resulting in 80-90% overall COD removal. GC/MS preliminary data indicate that the anaerobic pretreatment stage induced an initial structural conversion in the DNOC molecule, causing it to become more biodegradable by the aerobic microorganisms in the activated sludge process.

The initial breakdown of DNOC in the anaerobic pretreatment stage was due to co-metabolism. DNOC itself was toxic to the anaerobic microorganisms if no other, readily biodegradable, co-substrate was available to them. DNOC cannot be, therefore, used as a sole carbon source by the anaerobic bacteria. Sucrose has been used as the co-substrate in this study. The data suggest that there is a relationship between influent sucrose concentration and the performance of the anaerobic pretreatment stage in the conversion of DNOC. A ratio of sucrose to DNOC of 2:1 or higher resulted in a 95-100% conversion of DNOC in the anaerobic pretreatment stage. The anaerobic microorganisms failed to co-metabolize DNOC when the sucrose to DNOC influent concentration ratio was less than 2:1.

Toxicity test results show that effluent from the anaerobic pretreatment stage was more toxic to Microtox™ luminescent bacteria than the influent into the anaerobic pretreatment stage. This increase in toxicity was probably due to the accumulated anaerobic degradation by-products of sucrose and DNOC. However, after undergoing the aerobic treatment stage, the effluent from the activated sludge reactor was less toxic to the Microtox™ luminescent bacteria.

SECTION 3

RECOMMENDATIONS

1) It is recommended that additional investigations be performed to correlate effluent quality with influent loading changes and obtain more data concerning the kinetics of the reaction that is taking place in the anaerobic pretreatment process. This information would be used to test modifications in the operational hydraulic parameters of the anaerobic pretreatment process as well as the following aerobic treatment process in order to optimize the overall performance of the system.

2) Alternative, more economical co-substrates other than sucrose should be studied.

3) The performance of the anaerobic pretreatment process should be tested at lower operating temperatures, such as room temperature, in order to reduce energy requirements for operation.

4) It is recommended that this anaerobic-aerobic treatment process be applied to other priority pollutants, which have been found resistant to aerobic treatment, and their degradation-kinetics patterns be studied.

5) Overall treatment efficiency of DNOC and other aerobic-treatment-resistant priority pollutants should be tested in a two-stage anaerobic system.

6) Alternative anaerobic processes should be evaluated.

SECTION 4

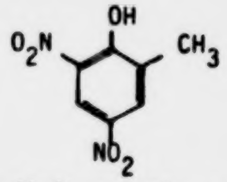
STATE-OF-THE-ART REVIEW

4,6-DINITRO-O-CRESOL CHARACTERIZATION

DNOC has been used as a herbicide for several decades. It is produced either by sulfonation of o-cresol followed by treatment with nitric acid or by treatment of o-cresol in glacial acetic acid with nitric acid at low temperature. Some important chemical and physical properties of DNOC are shown in Table 1. Due to its extensive use as a very effective herbicide, large numbers of investigations have been carried out to study its herbicidal mode of action as well as that of other nitrophenolic compounds. It has been established (58) that DNOC has an inhibitory effect on the assimilatory processes of soil microorganisms through uncoupling of the oxidative phosphorylation. Jensen (58,59), Jensen and Lautrup-Larsen (12), and Gundersen and Jensen (7) showed that the toxicity of DNOC is markedly pH dependent, namely an increase in the inhibitory effect of DNOC occurs with increasing acidity. It is generally assumed that the increased toxicity in low pH range is due to the greater permeability of the cytoplasm membrane to the undissociated molecules whose concentration depends on the hydrogen ion concentration. Since an increase in pH decreases the concentration of the undissociated DNOC there is a decrease in DNOC toxicity. At a pH range of 7.5-8.3, DNOC is the least toxic for most microorganisms (7,12). Van Rensen et al. (69,70) showed that DNOC is a potent inhibitor of photosynthesis, it strongly suppressed electron transport in isolated chloroplasts. Van Rensen and Wim (65) further demonstrated that DNOC acts as a competitive inhibitor of HCO_3^- binding in the photosynthetic electron transport in pea (Pisun sativum) isolated chloroplasts. Vlassak and Heremans (68) showed that DNOC inhibited soil nitrification. It also inhibited nitrogenase obtained from

TABLE 1. PHYSICAL AND CHEMICAL PROPERTIES OF 4,6-DINITRO-ORTHO-CRESOL (DNOC)*

Molecular formula: $C_6H_2(CH_3)OH(NO_2)_2$



4,6-dinitro-ortho-cresol
(DNOC)
CAS no. 534-52-1

Synonyms: 3,5-dinitro-o-cresol
3,5-dinitro-2-hydroxytoluene
2,4-dinitro-6-methylphenol
2-methyl-4,6-dinitrophenol
Antinonin, Dekrysil, Detal, Dinitrol, Ditrosol, Effusan, Elegetol, KIII, KIV, Lipan, Prokarbol, Selinon, Sinox

Molecular weight: 198.13

Appearance: yellow crystal

Melting point: 85.8°C

Vapor pressure: 0.000052 mm Hg at 20°C

Solubility: sparingly soluble in water
readily soluble in alkaline aqueous solutions, ether, acetohe, and alcohol (about 10%)

Log octanol/water partition coefficient: 2.85

pKa: 4.46
Henry's law constant: 1.4×10^{-6} atms·m^3·$mole^{-1}$

---

*Adapted from 2, 3, 4.

Azotobacter vinelandii, the inhibition was competitive, using STP as a substrate. Merenyuk and Timchenko (62) reported that an addition of 300 mg DNOC per kg soil reduced the counts of nitrifying and cellulytic bacteria.

Bringmann and Kühn (13,14) showed that DNOC starts to inhibit cell multiplication of Pseudomonas putida at the concentration of 16 mg/l, DNOC also inhibits degradation of glucose by P. fluorescens and by E. coli at 30 and 100 mg/l, respectively.

Ilivicky and Casida (71) in their work with mitochondria from various mammals and insects, and Popov, Velikii and Parkhomets (64) in their work with rat liver cells demonstrated that the inhibitory effect of DNOC is due to the uncoupling of oxidative phosphorylation.

ENVIRONMENTAL FATE OF DNOC

Aquatic Fate

Table 2 summarizes the aquatic fate data of DNOC. This pollutant will probably undergo slow photolytic destruction in ambient surface waters. Volatilization is not thought to be an important transport process, but DNOC might be strongly sorbed by clay minerals as indicated by its high octanol/water partition coefficient of log P=2.85. There is a possibility that DNOC could undergo hydrolysis while sorbed onto the clay structure. Although the biotransformation of DNOC has been demonstrated, it is uncertain whether biodegradation is an operational fate in ambient surface waters (4).

Fate in Soil

Biotransformation and biodegradation occur in soil and are probably the main processes of removal of DNOC from agricultural soils.

TABLE 2. SUMMARY OF AQUATIC FATE OF DNOC (4)

Environmental process	Summary statement	Confidence of data
Photolysis	Slow photooxidation may be the main aquatic fate pathway.	low
Oxidation	Displacement of the nitro-group by hydroxyl radical may be possible.	low
Hydrolysis	Hydrolysis might occur during sorption on a clay surface; insufficient information to assess this pathway.	low
Volatilization	Probably not important.	low
Sorption	This pollutant should be strongly sorbed by clay minerals.	low
Bioaccumulation	Importance is uncertain, due to its high toxicity.	low
Biotransformation/Biodegradation	May be very slow in ambient surface waters.	low

BIODEGRADATION OF DNOC

Degradation of DNOC by Soil Microorganisms

The decomposition of DNOC in soil has been studied by numerous investigators. Gunderson and Jensen (7) isolated from soil the bacteria Arthrobacter simplex (then called Corynebacterium simplex) that uses DNOC as nutrient in aerobic metabolism, with the formation of nitrite as metabolite. They found that DNOC concentration of 200 mg/l (at pH>7) appeared to be non-toxic. Nitrite begins to appear in the cultures after 2 to 3 days and approximately 70% of the DNOC—nitrogen is recovered as nitrite after 6 to 7 days. Increasing the DNOC concentration to 500 mg/l (at pH 8.0) resulted in a prolonged lag-time for nitrite production, but after 14 days 50% of the DNOC added was dissimilated. At 1,000 mg/l inhibition was appreciable and at 2,000 mg/l and 5,000 mg/l no growth or degradation of the compound took place. The authors suggested that the first degradation step of DNOC is the removal of the para-nitro group and its conversion to nitrite. They also suggested that the ring structure is subsequently ruptured, giving rise to aliphatic compounds which are in turn assimilated by the organism.

Tewfik and Evans (8) isolated from soil a pseudomonad which metabolized DNOC in pure cultures. The degradative pathway, as indicated by chemical isolation of intermediates, sequential induction, washed cell suspension experiments, and crude cell-free extracts, was shown to be as follows:

> 3,5-dinitro-o-cresol →3-amino-5-nitro-o-cresol →3-methyl-5-nitro-catechol →3-methyl-5-amino-catechol →2,3,5-trihydroxytoluene → ring cleavage

This pathway is different in its initial steps from that employed by Arthrobacter simplex, which, according to sequential induction evidence, follows the following route:

3,5-dinitro-o-cresol → 3-methyl-5-nitro-catechol →
2,3,5-trihydroxytoluene → ring cleavage

The authors concluded that the presence or absence of a nitroreductase, a different enzyme from nitrite reductase, is a determinant of the metabolic route by which the nitrogen of nitro-compounds is made available to the microorganisms.

Hamdi and Tewfik (9) showed that several species of *Rhizobinum* and *Azotobacter* and *Beijerinkia indica*, all soil bacteria, can actively degrade DNOC. They also determined that DNOC is reduced to 3-amino-5-nitro-o-cresol by *Rhizobinum leguminosarum*, in the early stages of its degradation.

Chambers and Kabler (10) found that 100 mg/l DNOC can be degraded by phenol-adapted cultures, but not degraded by cultures adapted to other tested phenolic derivatives. No percentage of removal was reported.

Tabak, Chambers and Kabler (11,57) extended the above investigation and tested the degradation of 104 phenolic compounds and aromatic hydrocarbons by phenol-adapted, aerobic soil bacteria. They observed loss of color and accumulation of nitrite in enrichment cultures with several aromatic nitro-compounds, including DNOC. Manometric experiments with the phenol-adapted bacteria (mainly *Pseudomonas* and *Flavobacterium*) as seed, showed only a slight oxygen uptake with 100 mg/l DNOC in comparison with the same concentration of o-cresol (7 percent in 3 hours) although loss of color indicated 60 percent decomposition of DNOC in the same time. When compared with the other 103 studied phenolic compounds, DNOC was classified by the authors as "resistant to degradation" due to its low oxygen consumption in Warburg tests, with phenol-adapted seed bacteria.

Jensen and Lautrup-Larsen (12) isolated from soils treated with 50-100 mg/l DNOC, two groups of aerobic bacteria capable of metabolizing DNOC.

Group I was comprised of seven strains of Arthrobacter-like organisms that grew very slowly and feebly. When DNOC served as sole substrate, some 18 to 28 percent of the DNOC-nitrogen was assimilated in cell substance as compared with 76-78 percent of the DNOC-nitrogen that was assimilated in cell substance when glucose was introduced as an additional carbon source. Group II was represented by two strains of Pseudomonads (possibly a species of Agrobacterium) that grew rapidly; 17-19 percent DNOC-N was assimilated in cell substance as cell-N and 46-47 percent was transformed into nitrite ion when DNOC served as sole substrate. No other nitrogenous metabolite other than nitrite was found. With glucose as a subsidiary carbon source, the nitrite formation was repressed and approximately 90 percent of the DNOC-N was found in the cell substance.

Wallnöefer et al. (66) investigated the transformation of DNOC by Azotobacter sp. isolated from agricultural soil, they found that in the absence of a nitrogen source, all strains of Azotobacter tested transformed DNOC to 6-acetamido-2-methyl-4-nitrophenol. The transformation was almost stoichiometric. In the presence of nitrogen source, bacterial growth was markedly inhibited and no transformation of the DNOC was observed. This demonstrates that there is a correlation between the reduction of DNOC in Azotobacter and the induction of the nitrogenase enzyme system, which is represented by ammonium or nitrate compounds.

Jensen (60), Merenyuk et al. (62), and Hurle et al. (63) demonstrated the importance of pretreatment and acclimation and showed that the rate of decomposition of DNOC in the soil that had been treated with the same compound was greater than the decomposition rate in a similar soil that had not been pretreated.

TREATABILITY AND TREATMENT ALTERNATIVES OF DNOC

Biological Treatment of DNOC

Tabak et al. (16) used a static-culture-flask biodegradation screening test to determine the biodegradability of 96 organic priority pollutants. They found that DNOC does not demonstrate significant bio-oxidation even after 28 days of incubation period under enrichment-culture conditions. The 5 and 10 mg/l DNOC samples were only degraded by 51 and 14%, respectively. Since the test was undertaken in favorable culture conditions, the actual removal performance in normal wastewater treatment conditions is expected to be even poorer.

Fuentes (1) studied the fate of DNOC in bench-scale activated sludge reactors using peptone and dextrose as main substrate to which acetone-dissolved DNOC was added. He concluded that the main removal mechanism for DNOC is biodegradation by microorganisms, whereas air stripping and adsorption-on-floc do not contribute to the removal of DNOC in activated sludge. Results of earlier short-term respirometer tests using unacclimated sludge showed that DNOC becomes growth inhibiter within 20-30 mg/l, and is toxic above 50 mg/l. For an influent DNOC concentration of less than 30 mg/l, the removal efficiencies are between 8 and 23.5% for a sludge-age-range of 3.5 to 9.5 days. The degradation of DNOC follows a zero-order kinetic rate.

Additional data on the treatability of DNOC in activated sludge (5) is given for synthetic wastewater as 58% removal and average achievable concentration of 2.1 mg/l.

Physical and Chemical Treatments of DNOC

Sedimentation--

A DNOC concentration of 0.19 mg/l was reported to be more than 95 percent removed from one coal mining effluent in a slurry pond (5).

Vakulenko and Marchenko (67) reported that coagulation experiments done on <4 mg/l DNOC with 10-25 mg/l $Al_2(SO_4)_3$ indicated that the maximum degree of removal was achieved at high concentrations of $Al_2(SO_4)_3$.

Activated Carbon Adsorption--

Dobbs and Cohen (17) reported activated carbon adsorption data of DNOC using Filtrasorb-300 granular activated carbon. The carbon dose required to reduce DNOC concentration from 10 mg/l to 1 mg/l at pH 5.2 is 53 mg/l. Hutton (52) reported that DNOC of 0.011 mg/l is more than 99 percent removed in a combined powdered activated carbon-activated sludge treatment.

Steam Stripping--

Treatability data for treating organic priority pollutants by steam stripping has been reported by Hwang and Fahrenthold (18). Due to its low activity coefficient (estimated as 308) and low vapor pressure, treatment of DNOC by steam stripping is unsuitable. The removal as calculated by the authors is less than 20% for steam stripping without reflux; even with 20 trays with reflux, the effluent DNOC concentration will still remain high.

ANAEROBIC DEGRADATION OF PHENOLIC AND NITROAROMATIC COMPOUNDS

Compared to the understanding of the aerobic degradation process, relatively little is known about the anaerobic degradation of most organic compounds. Published data of the anaerobic degradation of phenolic and nitroaromatic compounds are limited, and sometimes conflicting on the biodegradability of certain compounds.

Evans (47) reviewed the degradative mechanisms and routes of aromatic compounds in anaerobic environments and summarized them into the following three categories: a) photometabolism (by Athiorhodaceae), b) metabolism through nitrate respiration (by Pseudomonas and Moraxella sp.), and c) methanogenic fermentation by a consortium.

Healy and Young (48) studied the anaerobic degradation of 11 simple aromatic lignin derivatives: vanillin, vanillic acid, ferulic acid, cinnamic acid, benzoic acid, catechol, protocatechuic acid, phenol, p-hydroxybenzoic acid, syringic acid, and syringaldehyde. They found that all the tested compounds are biodegradable to methane and carbon dioxide with different acclimation periods ranging from two days for syringic acid to 21 days for catechol.

Hallas and Alexander (49) measured the transformation of mono and dinitro aromatic compounds in sewage effluent maintained under aerobic or anaerobic conditions. Most of the nitrobenzene, 3- and 4-nitrobenzoic acids, and 3- and 4-nitrotoluenes and much of the 1,2- and 1,3-dinitrobenzenes disappeared both in the presence and absence of oxygen. Under anaerobiosis, 2,6-dinitrotoluene and 3,5-dinitrobenzoic acid disappeared slowly, but no loss was evident in 28 days in aerated sewage. Aromatic amines did not accumulate during the aerobic decomposition of the mononitro compounds. They did appear in sewage incubated aerobically with the dinitro compounds and anaerobically with all the chemicals. Reduction of aromatic nitro group to amino group in anaerobic environment is again confirmed. Further treatment of those amino byproducts shows that anaerobic by-products of mononitro compounds disappear faster under aerobic than anaerobic conditions, whereas aerobic and anaerobic by-products of dinitro compounds resist aerobic and anaerobic microbial attack.

Recently, Boyd et al. (50) reported the anaerobic degradation of phenol and the ortho, meta, and para isomers of chlorophenol, methoxyphenol, and methylphenol (cresol), and nitrophenol in anaerobic sewage sludge. Eleven of the 12 monosubstituted phenols examined were degraded in anaerobic digester sludge. The times required for complete disappearance of phenol and the 12 monosubstituted phenols are given. The presence of Cl and NO_2 groups, especially the meta- and para-substitutes, inhibited methane generation. However, elimination or transformation of these substituents was accompanied by increased gas production. No obvious relationship between substituent position and susceptibility to anaerobic degradation was observed. Even though the transformation products of cresols were not observed directly, the authors state that the reduction of aromatic NO_2 groups to NH_2 in the anaerobic conditons almost certainly occurred.

GENERAL INFORMATION ON BIODEGRADATION PROCESSES OF ORGANIC COMPOUNDS

Biodegradation includes several different microbial processes: mineralization, detoxication, co-metabolism, activation, and defusing (20).

Mineralization is the conversion of an organic compound to inorganic products. When an organic molecule is mineralized, the transformation is usually the result of microbial action, and it is typically a growth-linked process.

Detoxification refers to the conversion of a toxicant to innocuous metabolite(s). Although mineralization is characteristically a detoxication, many other kinds of detoxication are known.

Co-metabolism is the metabolism of a compound that the organisms are not using as a nutrient. Co-metabolism does not result in mineralization; hence, organic products remain.

Activation takes place when a nontoxic molecule is converted to one that is toxic, or a molecule with low potency is made into a product of greater toxicity to some species. Activation also may occur during co-metabolism.

Defusing, the conversion of a potentially hazardous substance into an innocuous metabolite before the potential for harm is exerted, is so far known only in pure laboratory cultures.

Some of these types of reactions take place among the phenoxy herbicides. Thus, activation occurs when microorganisms convert 4-(2,4-dichlorophenoxy) butyric acid to 2,4-dichlorophenoxyacetic acid (2,4-D), because the latter but not the former is phytotoxic. Similarly, 2,4-dichlorophenoxyethanol sulfate is activated to 2,4-D. In turn, 2,4-D is detoxified as it is converted to 2,4-dichlorophenol but is mineralized when it is converted to CO_2. On the other hand, the removal of the butyrate moiety of 4-(2,4-dichlorophnoxy) butyrate to yield 2,4-dichlorophenol is an example of defusing since the herbicide, 2,4-D is not formed (20,21).

CO-METABOLISM

The phenomenon of co-metabolism, or sometimes called co-oxidation (22), was first reported by Leadbetter and Foster (23) when they noted the oxidation of (a) ethane to acetic acid and acetaldehyde, (b) propane to propionic acid and acetone, and (c) n-butane to n-butyric acid and 2-butanone during growth of Pseudomonas methanica on methane, the only hydrocarbon capable of supporting growth of the organisms.

Later, co-metabolism was defined by Foster (24) as follows: "Nongrowth hydrocarbons are oxidized when present as co-substrates in a medium in which one or more different hydrocarbons are furnished for growth."

Because of the discovery of the phenomenon of co-metabolism, many compounds that were once considered recalcitrant may actually undergo biodegradation. For example, the herbicide 2,4,5-trichlorophenoxyacetate (2,4,5-T) has been regarded as a recalcitrant compound because of repeated failure to isolate organisms capable of utilizing it as sole source of carbon and energy for growth (25). However, later research (26) indicated that 2,4,5-T could be oxidized by a co-metabolic process to the end product of 3,5-dichlorocatechol, which could then be co-metabolized (27,28) by another microbial species or completely metabolized (29).

The physiological basis for co-metabolism is not clear. Microorganisms are unable to proliferate on compounds as sole carbon source that they degrade by co-metabolism. The most likely hypothesis involves enzyme specificity. Many enzymes present in microbial cells catalyze reactions involving several different but chemically related substrates. While the inital enzyme characteristically catalyses its natural substrate to products that provide energy and a source of carbon for the active species, some products of the enzyme reactions may not be suitable for any other enzyme reaction in the culture and will subsequently accumulate (22,30).

Characterization and Identification of Co-metabolism

For organic compounds degraded by mineralizing conversion, an increase in population density of species is generally paralleled by an increase in degradation rate. Co-metabolism, however, is characterized by the lack of this increase in degradation rate because the bacterial species cannot use it for biosynthesis and have no growth benefits from the presence of the compound (20,30).

Generally, proof of disappearance of substrate and/or accumulation of end products and the inability of the microorganisms to utilize the studied compound as its sole source of carbon and energy for growth are required to clearly demonstrate a co-metabolic process (32).

Enhancement of Co-metabolism--

Co-metabolism characteristically leads to a slow rate of products formation if the number of cells bearing the responsible enzymes is small. A number of techniques have been reported to enhance the rate of co-metabolism.

Analog enrichment technique -- The effect of co-substrate was observed by Broadbent and Norman (33), soil organic matter became a better source of nutrient for the microbial soil population when readily decomposable organic matter was added, the authors did not offer an explanation for this phenomenon.

Horvath (34), studying the co-metabolism of 2,3,6-trichlorobenzoate, demonstrated that microorganisms capable of a co-metabolic degradation of organic pollutants can be enriched by application of biodegradable analogues of the pollutant to the microbial ecosystem. Horvath (32) indicated that the technique of "analogue enrichment" also accounts for the enhanced rate of decomposition of organic matter described by Broadbent and Norman (33).

Co-substrate enrichment technique -- Later, a structurally unrelated substance, glucose, was used as an enrichment agent for the study of the co-metabolism of chlorobenzoates (35,36). The results showed that employment of "co-substrate enrichment technique", considerably reduced the time required for complete degradation of the studied chlorobenzoates. Horvath (35) concludes that the addition of the co-substrate serves primarily to increase the numbers of microorganisms capable of affecting an oxidation of the halogenated compound and does not necessarily induce a specific microbial population.

Glucose has been reported to be an effective co-substrate for decomposition of vanillin (37) and p-anisidine (38) by soil microorganisms, alkyl benzene sulfonate by Pseudomanas sp. (39,40), and diazinon by Arthrobacter sp. (41). The wide range of compounds for which glucose serves as co-substrate supports the function of the co-substrate proposed above by Horvath (35).

However, in some studies glucose was reported to be an inferior co-substrate. In a study of the co-metabolism of cyclohexane by Beam and Perry (42), the addition of hexadecane as co-substrate resulted in a significantly higher rate of co-metabolic oxidation than with an equal amount of glucose as co-substrate. The authors proposed that cyclohexane, unlike compounds such as chlorobenzoates (35), may lack the capacity to induce the requisite oxidative enzymes responsible for its degradation. The addition of glucose, which is oxidized by pathways not involving an oxygenase, thus did not help in inducing the oxygenase (43).

In another study on the effect of various co-substrates on the rate of co-metabolic oxidation of malathion (o,o-dimethyl-S-(1,2-dicarbethoxy) ethy phosphorodithionate) in soil (44), n-heptadecane was found to be the most effective co-substrate. The addition of glucose, acetate and many other co-substrates did not have an appreciable effect on the oxidation rate. The same co-substrates were also tested for their effect on the oxidation of DDT, lindane and dieldrin; however, no degradation of these compounds was observed.

DiGeronimo, Nikaido and Alexander (45) compared structural analogues and glucose as co-substrates and, in contrast to the finding by Horvath (34,35,36), observed inappreciable degradations of chlorobenzoates, when using the same techniques as Horvath except for inoculation with sewage instead of pure cultures.

Increased density of organisms technique -- Jacobson, O'Mara and Alexander (46) demonstrated the co-metabolism of several herbicides in sewage. The use of concentrated sewage microflora as seed greatly enhanced the rate of the co-metabolic conversion compared with the rate of conversion with unconcentrated seed.

SECTION 5

EXPERIMENTAL

To achieve the objectives of this investigation the experimental study was divided into two main parts;

I. Anaerobic batch tests

II. Anaerobic-aerobic pilot plant applications.

I. ANAEROBIC BATCH TESTS

Two anaerobic batch testing techniques were used:

1. shaker bottle tests
2. continuously-stirred tests

Detailed information about these two testing techniques is given in Appendix A.

The anaerobic batch test studies were designed to provide data on:

a. The biodegradability of DNOC in anaerobic conditions in the presence of an additional carbon source (co-substrate).

b. The kinetics of the anaerobic biodegradation of DNOC.

c. The toxicity changes that result from the anaerobic biodegradation of DNOC.

a. The Biodegradability of DNOC in Anaerobic Conditions in the Presence of an Additional Carbon Source (co-substrate).

Early continuously-stirred batch tests results (see Section 6) indicated that DNOC at a concentration of 100 mg/l will not biodegrade in anaerobic conditions unless an additional carbon source is provided. Sucrose was chosen as the cosubstrate, since it is readily biodegradable. The correlation between sucrose concentration and the biodegradation of DNOC in anaerobic conditions was established. Filtered and unfiltered domestic primary sludge were also tested as possible, more economically feasible co-substrates.

b. The Kinetics of the Anaerobic Biodegradation of DNOC

Shaker bottle tests and continuously-stirred tests using varying combinations of initial DNOC concentration and initial sucrose concentration were performed. Samples were taken every 24 hours for the duration of the test, usually 7 days, and were analyzed for pH, oxidation-reduction potential (ORP), suspended solids (SS), volatile suspended solids (VSS), soluble chemical oxygen demand (CODs), soluble total organic carbon (TOC), and total organic acids (TOA). Samples were also taken for determination of DNOC concentration, as well as sucrose concentration (details are in Appendix C).

c. The Toxicity Changes that Result from the Anaerobic Biodegradation of DNOC.

It was important to determine whether the anaerobic biodegradation of DNOC resulted in by-product(s) having toxicity characteristics different from the toxicity characteristics of the original compound. In order to determine any toxicity changes due to the anaerobic biodegradation of DNOC, daily samples were taken from the shaker bottle batch tests and from the continuously-stirred batch tests and subjected to toxicity analysis using the Beckman Microtox^{TM} toxicity analyzer system. The method for carrying out the toxicity analysis is outlined in Appendix C.

II. ANAEROBIC-AEROBIC PILOT PLANT APPLICATIONS

The anaerobic-aerobic pilot plant was set up in order to:

a. test the hypothesis that pretreatment of DNOC under anaerobic conditions will result in bioconversion-products, or biodegradation-products, which will be more readily biodegradable than DNOC in the activated sludge process; namely, to test the impact of biological anaerobic pretreatment of DNOC on the efficiency of the overall removal of DNOC in the activated sludge process.

b. provide data on the co-metabolism of DNOC as a function of the concentration of an additional, more readily biodegradable, carbon source (sucrose in this case) in the anaerobic pretreatment stage.

c. provide data on the toxicity changes that result from the anaerobic pretreatment of DNOC.

d. provide data on some hydraulic design parameters for an optimized performance of the anaerobic pretreatment filter column.

Pilot Plant Apparatus

The pilot plant consisted of one anaerobic upflow sand filter and one activated sludge tank, as shown in Figure 1. The anaerobic upflow sand filter consisted of a plexiglass column 8 feet high. Its lower section was 5 feet high and 3 inch ID and its upper section was 3 feet high and 4.25 inch ID. Prewashed sand, which was used as matrix for the attachment of microorganisms, was 3.0 feet high in the lower section. When operating, the bed expanded about 30% to a height of 4.0 feet. The temperature of the column was maintained at 36.5°C by heating tapes surrounding the column. The temperature was controlled by a Versa-therm proportional temperature controller (Cole-Parmer Inst.) with a thermister sensor inserted into the column.

The anaerobic upflow sand filter column was operated as a flow through system with a total internal flow rate maintained at about 3,000 l/day. Sodium chloride tracer tests showed that the liquid phase was completely mixed (Figures 2 and 3). However, the distribution of the biomass in the fluidized bed was uneven (Figure 4). The microorganisms were predominately concentrated in the upper section of the sand bed. The pattern of the biomass distribution in the column bed resulted in a distinct dividing plane formed between the sand particles in the lower section of the bed which were sparsely populated with biomass, and the sand particles in the upper section of the bed which

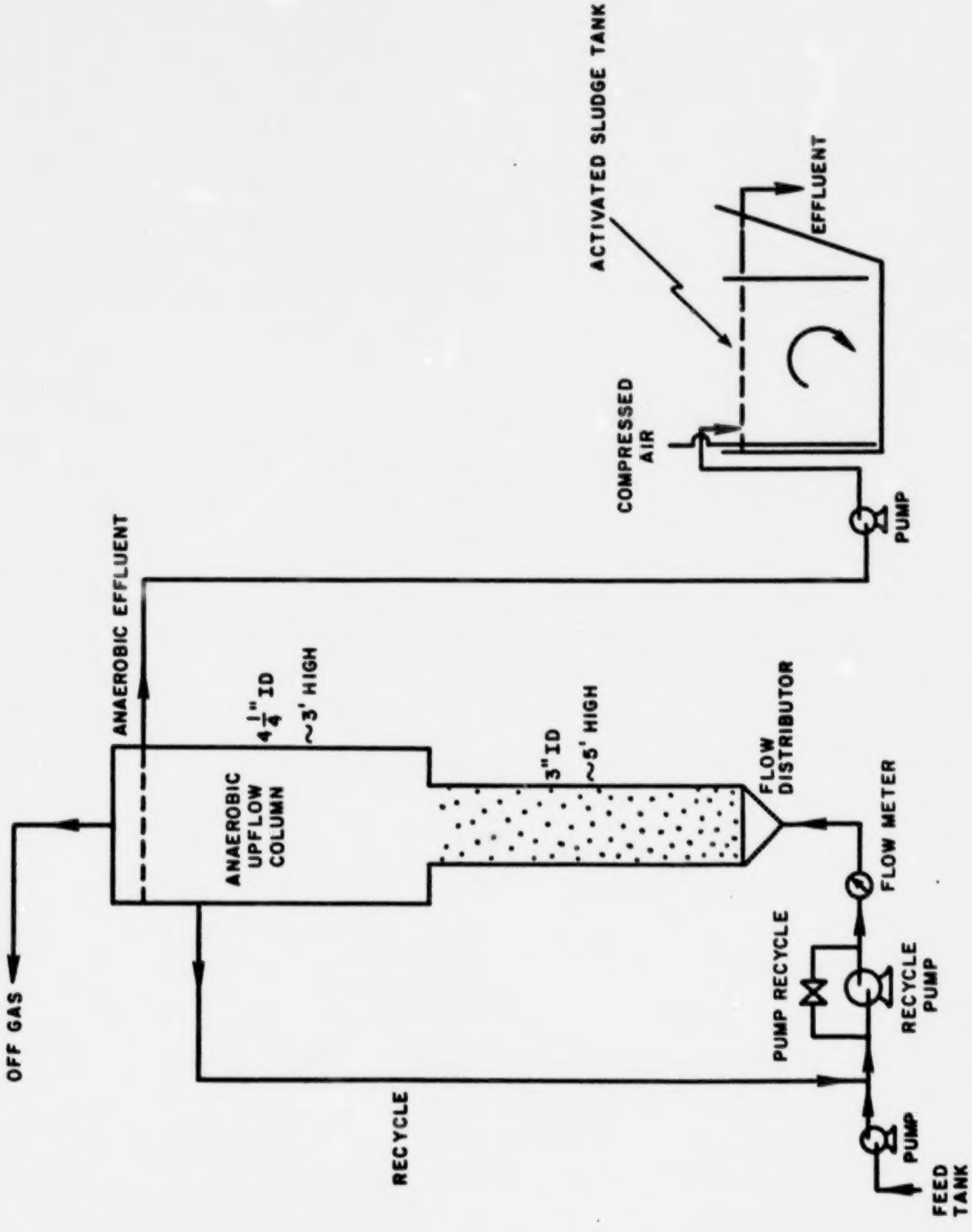

OFF GAS
RECYCLE
ANAEROBIC UPFLOW COLUMN
ANAEROBIC EFFLUENT
4 1/4" ID
~3' HIGH
3" ID
~5' HIGH
FLOW DISTRIBUTOR
FLOW METER
PUMP RECYCLE
RECYCLE PUMP
PUMP
FEED TANK
PUMP
COMPRESSED AIR
ACTIVATED SLUDGE TANK
EFFLUENT

Figure 1. Schematic of pilot plant apparatus.

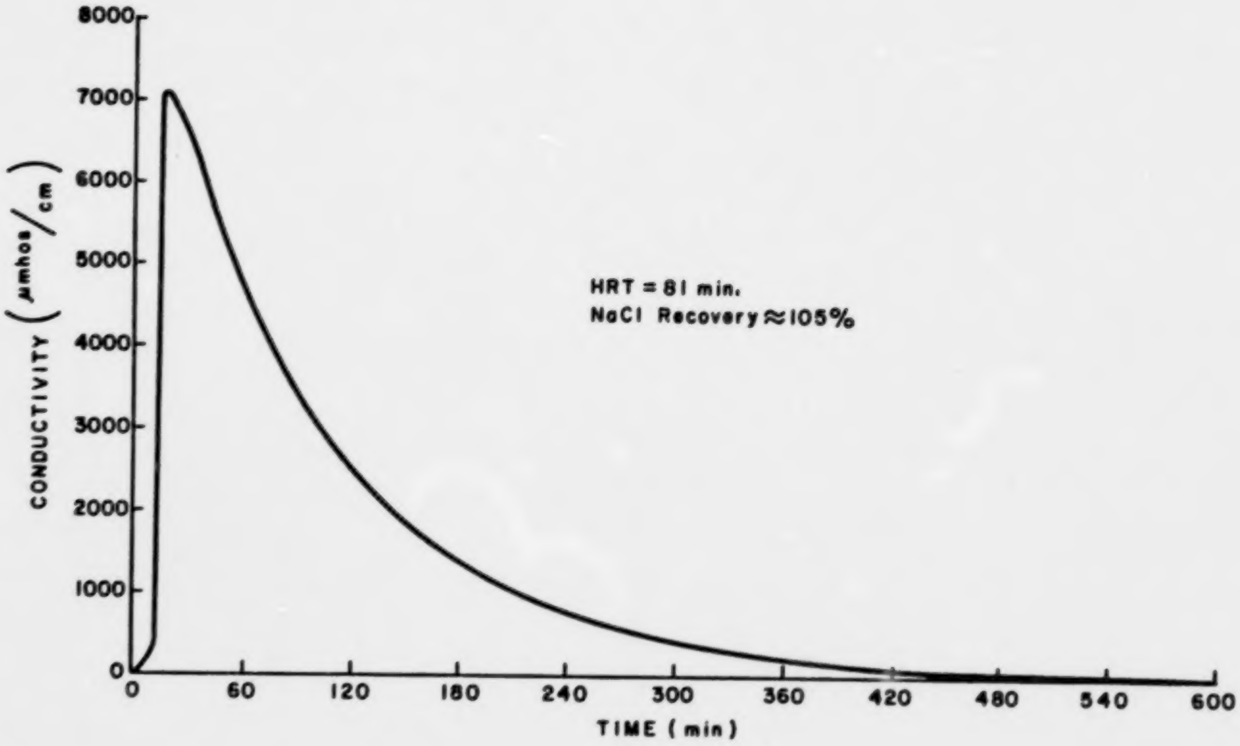


Figure 2. Sodium chloride (NaCl) tracer curve for the anaerobic column.

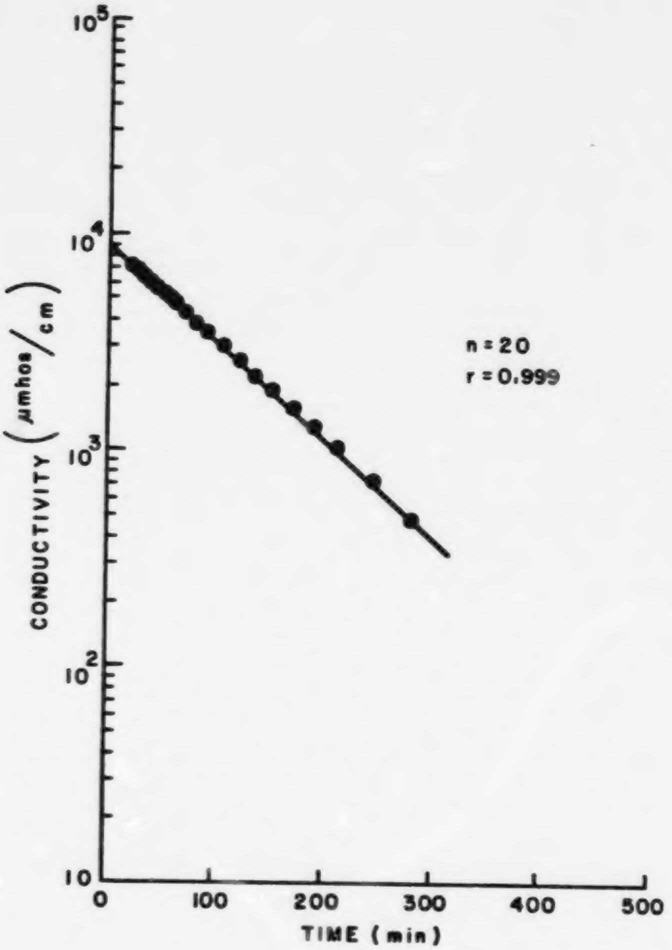


Figure 3. A linearized NaCl tracer curve for the anaerobic column reflecting a first-order mixing in the column.

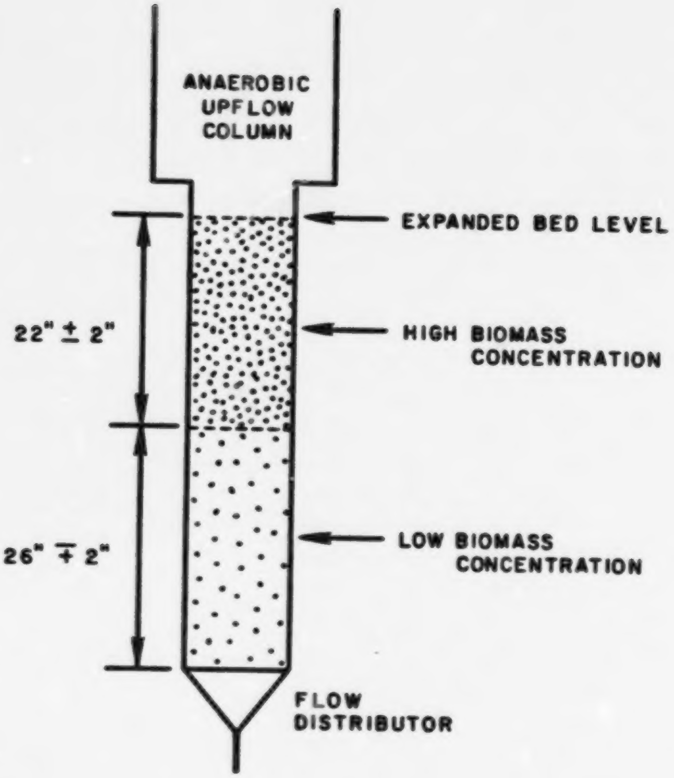

ANAEROBIC
UPFLOW
COLUMN
EXPANDED BED LEVEL
22" ± 2"
HIGH BIOMASS CONCENTRATION
26" ∓ 2"
LOW BIOMASS CONCENTRATION
FLOW DISTRIBUTOR

Figure 4. The distribution of biomass in the anaerobic column.

were densely populated with biomass. The biomass distribution in the fluidized bed appears in Table 3.

TABLE 3. BIOMASS DISTRIBUTION IN THE FLUIDIZED BED

Sample Source	VSS (mg/l)
Anaerobic bed upper section	10,700 - 11,300
Anaerobic bed lower section	1,940 - 3,050
Anaerobic column effluent	12 - 104

The activated sludge tank was the conventional 19 liters plexiglass tank with a 14 liter aeration compartment and a 5 liter clarification compartment. Effluent from the anaerobic upflow column was pumped directly into the activated sludge unit as feed.

The anaerobic filter column was started up by several inoculations with seed from a local municipal sewage treatment plant sludge digester. It was then operated during an acclimation period and throughout the testing period as a flow through system. Loading concentrations of the different components of the influent were carefully controlled. The anaerobic filter column effluent and the activated sludge effluent were monitored, monitoring details are in Appendix A.

SECTION 6

RESULTS AND DISCUSSION

The results of the investigation are summarized as follows:

A) The removal of DNOC under anaerobic conditions;

B) The co-metabolism of DNOC under anaerobic conditions;

C) The removal of COD in the anaerobic-aerobic system;

D) The changes in toxicity due to anaerobic-aerobic treatment.

A) THE REMOVAL OF DNOC UNDER ANAEROBIC CONDITIONS

DNOC Removal in a Batch and a Flow-Through Anaerobic Reactor

Preliminary shaker bottle batch tests results (Table 4) in which sucrose (3.0 g/l) was used as a co-substrate clearly indicated that DNOC was degradable under anaerobic conditions. Over a period of 7 days, the original DNOC concentration of 100 mg/l was reduced to 15 mg/l. The reduction in the soluble COD and TOC was only 16% and 22%, respectively, which was coupled by an increase in TOA from 370 mg/l at day 0 to 2,260 mg/l at day 7.

Results (Table 5) from the shaker bottle batch tests in which filtered primary sludge was used as a co-substrate also provided supporting evidence that DNOC is anaerobically degradable. The DNOC concentration decreased from 92 mg/l in day 0 to 53 mg/l in day 7.

Anaerobic continuously-stirred batch tests (Table 6) confirmed the results of the anaerobic shake bottle tests. The original concentration of 100 mg/l of DNOC was reduced to 0.2 mg/l over a period of 7 days in the presence of 3.0 g/l sucrose as a co-substrate. The decrease in soluble COD was 11% over a period of 7 days while the TOA level increased from 318 mg/l at day 0 to 2,600 mg/l at day 7.

DNOC was treated in the anaerobic-aerobic system (Tables 7 and 8)

TABLE 4. ANAEROBIC SHAKER BOTTLE BATCH TEST RESULTS

Time (days)	Sample*	pH	SS (mg/l)	VSS (mg/l)	TOA (mg/l)	CODs (mg/l)	TOC (mg/l)	DNOC (mg/l)	EC 50*** (5 min)	EC 50 (15 min)
0	S	8.43	138	64.0	341	3130	1720	--	NTO**	NTO
	D	8.54	142	69.0	371	3340	1780	100	10.1%	7.16%
1	S	6.84	279	197	2150	2550	1430	--	NTO	NTO
	D	6.89	246	153	1950	2810	1610	78.6	9.62%	7.82%
2	S	6.82	245	177	2210	2600	1390	--	NTO	NTO
	D	6.84	188	123	2230	2780	1530	61.2	17.6%	14.5%
3	S	6.70	203	145	2310	2630	1490	--	NTO	NTO
	D	6.79	172	115	2160	2790	1780	39.8	25.2%	23.4%
4	S	6.77	234	156	2700	2560	1360	--	40.9%	8.92%
	D	6.85	172	114	2180	2710	1400	21.9	21.2%	14.5%
5	S	6.75	130	111	--	2850	1660	--	9.97%	9.06%
	D	6.81	89.5	68.5	--	2980	1660	18.0	22.5%	17.8%
6	S	6.76	114	93.0	--	3100	1750	--	56.4%	37.7%
	D	6.82	143	86.5	2170	3240	1810	15.5	23.4%	15.7%
7	S	6.76	88.0	73.5	2600	2790	1320	--	33.5%	23.2%
	D	6.83	80.0	56.5	2260	2820	1390	14.6	23.0%	18.2%

*S - Sucrose Only (3.0 g/l); D - Sucrose (3.0 g/l) + DNOC (100 mg/l)

**No Toxicity Observed

***EC_{50} is expressed in units of % of initial sample concentration.

TABLE 5. RESULTS OF SHAKER BOTTLE BATCH TEST USING FILTERED PRIMARY SLUDGE AS CO-SUBSTRATE

Time (days)	pH	SS (mg/l)	VSS (mg/l)	CODs (mg/l)	Residual DNOC* (mg/l)
0	7.3	130	95	579	92
7	7.2	195	145	630	53
13	7.3	145	90	674	50

*Initial DNOC concentration = 100 mg/l.

TABLE 6. THE EFFECT OF SUCROSE CONCENTRATION ON THE CO-METABOLISM OF DNOC IN CONTINUOUSLY-STIRRED BATCH TEST*

Sample	Time (days)	pH	SS (mg/l)	VSS (mg/l)	TOA (mg/l)	CODs (mg/l)	DNOC (mg/l)	Sucrose (g/l)
A	0	8.0	74	28	278	3440	--	3.0
	1/2	7.8	101	53	297	3500	--	--
	1	6.8	237	184	2950	3130	--	0
	1 1/2	6.9	208	162	--	3100	--	--
	2	6.9	192	143	2670	3170	--	0
	3	6.9	196	164	--	3240	--	0
	4	6.9	144	127	2440	3040	--	0
	5	7.0	103	84	--	3020	--	0
	6	7.0	62	48	--	3020	--	0
	7	7.0	116	86	2460	3070	--	0
B	0	7.9	54	20	318	3590	100	3.0
	1/2	7.9	77	42	309	3650	--	--
	1	6.9	185	140	2200	3340	75.1	0
	1 1/2	6.9	176	135	--	3140	--	--
	2	6.9	157	118	2680	3140	46.5	0
	3	6.9	158	128	--	3370	16.9	0
	4	6.9	157	126	2480	3210	4.4	0
	5	6.9	132	101	--	3300	0.8	0
	6	6.9	140	114	--	3180	0.5	0
	7	6.9	147	120	2600	3210	0.2	0
E	0	7.9	51	17	320	639	100	3.0
	1/2	7.9	56	20	274	610	--	--
	1	7.9	64	20	446	656	--	0
	1 1/2	7.9	74	28	--	570	--	--
	2	7.9	55	13	280	559	95.0	0
	3	7.9	62	22	--	--	--	0
	4	7.9	53	20	228	541	--	0
	5	7.9	48	10	--	525	101	0
	6	7.9	54	18	--	508	98.7	0
	7	7.9	93	31	220	491	102	0

*Initial Concentrations:

Sample	DNOC (mg/l)	Sucrose (g/l)	$NaHCO_3$ (g/l)	$(NH_4)_2HPO_4$ (g/l)
A	0	3.0	10.7	1.7
B	100	3.0	10.7	1.7
E	100	0	10.7	1.7

TABLE 7. THE PERFORMANCE OF THE ANAEROBIC COLUMN

No.	Feed Conc. DNOC (mg/l)	Feed Conc. Sucrose (g/l)	Influent Q (l/day)	Influent HRT* (days)	Effluent pH	Effluent ORP (MV)	Effluent Alkalinity (mg/l as $CaCO_3$)	Effluent SS (mg/l)	Effluent VSS (mg/l)	Effluent DNOC (mg/l)	Effluent Sucrose (g/l)	Influent COD (mg/l)	Influent TOC (mg/l)	Effluent CODs (mg/l)	Effluent TOC (mg/l)	Average gas production (l/day)
1	10.0	3.0	4.0	3.0	7.0	-300	3300			N.D.						2.0
2	20.0	3.0	4.0	3.0	7.0	-290	3300	52	43	N.D.		3200	--	2000	--	2.4
3	30.0	3.0	4.0	3.0	7.0	-290	3300	110	86	N.D.		3200	--	2350	--	1.6
4	50.0	3.0	4.0	3.0	6.9	-290	3400			N.D.		3200	--	2500	--	0.8
5	75.0	3.0	4.0	3.0						N.D.		3300	--	2800	--	0.75
6	100.	3.0	4.0	3.0						N.D.						0.7
7	125	3.0	4.0	3.0						N.D.						0.64
8	150	3.0	4.0	3.0						N.D.						0.4
9	200	3.0	4.0	3.0						N.D.						0.35
10	250	3.0	4.0	3.0						N.D.						0.32
11	325	3.0	4.0	3.0						N.D.						0.29
12	400	3.0	4.0	3.0						N.D.						0.19
13	500	3.0	4.0	3.0	6.4	-340	3400	77	66	N.D.		4070	1500	2960	1300	0.19
14	600	3.0	4.0	3.0	6.4	-280				N.D.						
15	500	3.0	4.0	3.0						N.D.						
16	500	3.0	4.0	3.0	6.4	-340	3000	100	82	N.D.		4070	1500	2850	1220	
17	500	2.0	4.0	3.0						N.D.						
18	500	2.0	4.0	3.0	6.3	-310	2600	110	93	N.D.		2950	1120	2170	705	
19	500	1.5	4.0	3.0						0.45						
20	500	1.5	4.0	3.0	6.3	-290	2000	72	61	0.55		2390	882	1670	605	
21	500	1.0	4.0	3.0	6.3	-160	1500			2.17						
22	500	1.0	4.0	3.0	6.3	-150	1500			3.11						
23	500	1.0	4.0	3.0						2.15		1820	704	1260	585	
24	500	0.75	4.0	3.0	6.4	-120	1200	77	71	46.7						
25	500	0.75	4.0	3.0						55.5		1540	590	1170	444	
26	500	0.75	4.0	3.0						101		1540	590	1240	494	
27	500	0.75	4.0	3.0	6.4	0	1100	14	12	104						
28	500	1.5	4.0	3.0						62.8						

TABLE 7. Cont.

No.	Feed Conc. DNOC (mg/l)	Feed Conc. Sucrose (g/l)	Influent Q (l/day)	Influent HRT* (days)	Effluent pH	Effluent ORP (MV)	Effluent Alkalinity (mg/l as $CaCO_3$)	Effluent SS (mg/l)	Effluent VSS (mg/l)	Effluent DNOC (mg/l)	Effluent Sucrose (g/l)	Influent COD (mg/l)	Influent TOC (mg/l)	Effluent CODs (mg/l)	Effluent TOC (mg/l)	Average gas production (l/day)
29	500	1.5	4.0	3.0	6.3	-70				49.5						
30	500	1.5	4.0	3.0	6.3	-90	2100			42.2						
31	500	1.5	4.0	3.0						32.7						
32	500	1.5	4.0	3.0						10.7						
33	500	1.5	4.0	3.0						7.24						
34	500	1.5	4.0	3.0	6.2	-160	2100	64	58	4.01						
35	500	1.5	4.0	3.0						2.68						
36	500	1.5	4.0	3.0						3.41						
37	500	1.5	4.0	3.0						2.08						
38	500	1.5	4.0	3.0				77	68	2.01		2390	882	1870	522	
39	600	1.5	4.0	3.0						5.87						
40	750	2.0	4.0	3.0	6.2	-160				2.68						
41	750	2.0	4.0	3.0	6.2	-150	2000			14.7						
42	750	2.0	4.0	3.0				115	104	87.8						
43	750	2.0	4.0	3.0						206		3300	1400	2430	687	
44	750	3.0	4.0	3.0	6.5	+20	2500	76	67	318		4240	1780	3260	1030	
45	250	3.0	4.0	3.0	6.2	-190	2300			0.95						
46	250	2.0	4.0	3.0						0.48						
47	250	2.0	4.0	3.0	6.1	-270	1900	105	95	N.D.		2490	846	2410	590	
48	250	2.0	4.8	2.5						0.64						
49	250	2.0	4.8	2.5	6.1	-270	1800	41	35	0.54		2490	846	2100	490	
50	250	2.0	6.0	2.0						0.25						
51	250	2.0	6.0	2.0	6.1	-280	1700	55	44	0.25		2490	846	1980	466	
52	250	2.0	7.5	1.6						0.21						
53	250	2.0	7.5	1.6	6.1	-280	1700			0.24		2490	846	1950	480	
54	250	2.0	10	1.2				86	79	N.D.						
55	250	2.0	10	1.2						N.D.						

TABLE 7. Cont.

	Feed Conc.		Influent		Effluent							Influent		Effluent		
No.	DNOC (mg/1)	Sucrose (g/1)	Q (1/day)	HRT* (days)	pH	ORP (MV)	Alkalinity (mg/1 as $CaCO_3$)	SS (mg/1)	VSS (mg/1)	DNOC (mg/1)	Sucrose (g/1)	COD (mg/1)	TOC (mg/1)	CODs (mg/1)	TOC (mg/1)	Average gas production (1/day)
56	250	2.0	13	0.93						N.D.						
57	250	2.0	13	0.93	6.1	-270	1700			N.D.	0.24	2490	846	1780	450	
58	250	2.0	17	0.72						0.01						
59	250	2.0	17	0.72						0.60						
60	250	2.0	17	0.72	6.1	-260	1700	79	70	0.12	0.24	2490	846	1870	518	
61	250	2.0	21	0.58						0.08						
62	250	2.0	21	0.58						0.05						
63	250	2.0	21	0.58	6.1	-270	1700	85	76	0.14	0.24	2490	846	1800	450	
64	250	2.0	28	0.43						N.D.						
65	250	2.0	28	0.43						0.12						
66	250	2.0	28	0.43	6.1	-270	1700			0.09	0.24	2490	846	1880	425	3.8
67	250	2.0	43	0.28						0.02						
68	250	2.0	43	0.28	6.1	-270	1700	87	61	0.03	0.22	2490	846	1980	500	8.2
69	250	2.0	72	0.17						0.06						
70	250	2.0	72	0.17	6.1	-270	1700	54	45	N.D.	0.22	2490	846	1840	548	9.2
71	250	2.0	26	0.47						N.D.						
72	250	2.0	26	0.47	6.1	-280	1700			N.D.	0.24	2490	846	1910	562	--
73	250	1.0	26	0.47						1.03						
74	250	1.0	26	0.47	6.1	-230	1000	56	45	1.38	0.24	1290	421	1100	288	0.2
75	250	0.5	26	0.47	6.1	-140				14.0						
76	250	0.5	26	0.47	6.1	-120				19.9		850	291	733	215	
77	250	0.5	26	0.47	6.1	-79	580	42	35	21.0	0.22	850	291	707	229	
78	250	0.25	26	0.47	6.1	-67				61.8						
79	250	0.25	26	0.47						112						
80	250	0.25	26	0.47	6.1	+7	420	25	21	126	0.20	621	135	557	167	
81	250	0.25	26	0.47						133						
82	0	0.25	26	0.47			1600	47	42	--		2200	654	1920	522	

*HRT is based on influent flow rate, Q.

TABLE 8. THE PERFORMANCE OF THE ACTIVATED SLUDGE REACTOR

No.	Feed to the system		Flow Rate (1/day)	Reactor			Influent			Effluent	
	DNOC (mg/1)	Sucrose (g/1)		pH	MLSS (mg/1)	MLVSS (mg/1)	DNOC (mg/1)	CODs (mg/1)	TOC (mg/1)	CODs (mg/1)	TOC (mg/1)
1	10.0	3.0	4.0				N.D.				
2	20.0	3.0	4.0	8.5	1800	1500	N.D.	2000	--	80	--
3	30.0	3.0	4.0	8.2	2000	1600	N.D.	2350	--	70	--
4	50.0	3.0	4.0	7.9	2400	2000	N.D.	2500	--	65	--
5	75.0	3.0	4.0	8.6	2800	2100	N.D.	2800	--	80	--
6	100	3.0	4.0				N.D.				
7	125	3.0	4.0				N.D.				
8	150	3.0	4.0				N.D.				
9	200	3.0	4.0				N.D.				
10	250	3.0	4.0				N.D.				
11	325	3.0	4.0				N.D.				
12	400	3.0	4.0				N.D.				
13	500	3.0	4.0				N.D.	2960	1300	386	--
14	600	3.0	4.0				N.D.				
15	500	3.0	4.0				N.D.				
16	500	3.0	4.0	8.8	2000	1200	N.D.	2850	1220	460	--
17	500	2.0	4.0				N.D.				
18	500	2.0	4.0		1500	850	N.D.	2170	705	440	130
19	500	1.5	4.0				0.45				
20	500	1.5	4.0		1400	780	0.55	1670	605	420	175
21	500	1.0	4.0				2.17				
22	500	1.0	4.0				3.11				
23	500	1.0	4.0		1100	570	2.15	1260	585	380	180
24	500	0.75	4.0				46.7				
25	500	0.75	4.0				55.5	1170	444	484	146
26	500	0.75	4.0		1100	580	101	1240	494	522	158
27	500	0.75	4.0		1100	520	104				
28	500	1.5	4.0				62.8				

TABLE 8. Cont.

	Feed to the system		Flow	Reactor			Influent			Effluent	
No.	DNOC (mg/1)	Sucrose (g/1)	Rate (1/day)	pH	MLSS (mg/1)	MLVSS (mg/1)	DNOC (mg/1)	CODs (mg/1)	TOC (mg/1)	CODs (mg/1)	TOC (mg/1)
29	500	1.5	4.0				49.5				
30	500	1.5	4.0				42.2				
31	500	1.5	4.0				32.7				
32	500	1.5	4.0				10.7				
33	500	1.5	4.0				7.24				
34	500	1.5	4.0	8.5	250	220	4.01				
35	500	1.5	4.0				2.68				
36	500	1.5	4.0				3.41				
37	500	1.5	4.0				2.08				
38	500	1.5	4.0				2.01	1870	522	510	215
39	600	1.5	4.0				5.87				
40	750	2.0	4.0				2.68				
41	750	2.0	4.0				14.7				
42	750	2.0	4.0	8.9	450	390	87.8				
43	750	2.0	4.0				206	2430	687	475	240
44	750	3.0	4.0		1180	970	318	3260	1030	700	185
45	250	3.0	4.0				0.95				
46	250	2.0	4.0				0.48				
47	250	2.0	4.0	8.6	1100	920	N.D.	2410	590	425	111
48	250	2.0	4.8				0.64				
49	250	2.0	4.8		1000	860	0.54	2100	490	310	68
50	250	2.0	6.0				0.25				
51	250	2.0	6.0		1400	1160	0.25	1980	466	230	36
52	250	2.0	7.5				0.21				
53	250	2.0	7.5	8.5	1770	1440	0.54	1950	480	220	30
54	250	2.0	10				N.D.				
55	250	2.0	10				N.D.				

TABLE 8. Cont.

	Feed to the system		Flow	Reactor			Influent			Effluent	
No.	DNOC (mg/1)	Sucrose (g/1)	Rate (1/day)	pH	MLSS (mg/1)	MLVSS (mg/1)	DNOC (mg/1)	CODs (mg/1)	TOC (mg/1)	CODs (mg/1)	TOC (mg/1)
56	250	2.0	13				N.D.				
57	250	2.0	13				N.D.	1780	450	--	--
58	250	2.0	17				0.01				
59	250	2.0	17				0.60				
60	250	2.0	17		2300	1700	0.12	1870	518	230	43
61	250	2.0	21				0.08				
62	250	2.0	21				0.05				
63	250	2.0	21				0.14	1800	450	--	--
64	250	2.0	28				N.D.				
65	250	2.0	28				0.12				
66	250	2.0	28				0.09	1880	425	220	33
67	250	2.0	43				0.02				
68	250	2.0	43	8.5	3700	2500	0.03	1980	500	755	245
69	250	2.0	72				0.06				
70	250	2.0	72				N.D.	1840	548	1720	512
71	250	2.0	26				N.D.				
72	250	2.0	26		2200	1860	N.D.	1910	562	385	200
73	250	1.0	26				1.03				
74	250	1.0	26				1.38	1100	288	180	26
75	250	0.5	26				14.0				
76	250	0.5	26				19.9	733	215	--	--
77	250	0.5	26	8.2	1750	1100	21.0	707	229	245	56
78	250	0.25	26				61.8				
79	250	0.25	26				112				
80	250	0.25	26				126	557	167	293	70
81	250	0.25	26				133				
82	0	0.25	26				--	1920	522	94	42

over influent concentration range of 10 mg/l to 750 mg/l. As shown in Table 9, the DNOC was not detected in the anaerobic column effluent with DNOC concentrations as high as 600 mg/l, when the sucrose concentration in the influent was maintained at 3.0 g/l and the hydraulic retention time (HRT) at 3 days.

The Effect of Hydraulic Retention Time on the Removal of DNOC Under Anaerobic Conditions

The effect of the HRT on the performance of the anaerobic column was determined under the following conditions:

sucrose concentration = 2.0 g/l
DNOC concentration = 250 mg/l
HRT = 0.17 to 3.0 days

As seen in Table 10, the removal of DNOC by the anaerobic column was > 99.7% even for HRT as short as 0.17 days. Hence, under these given operational conditions, no effect of HRT on the removal of DNOC was observed. However, the anaerobic gas production was increased (Table 7 runs No. 66-70) when the HRT decreased at constant influent concentration.

The By-products Resulting From the Removal of DNOC Under Aanaerobic Conditions

Acid extracted samples (Appendix C) from anaerobic shaker bottle test, anaerobic continuously-stirred batch test, anaerobic column effluent and activated sludge reactor effluent were analyzed by GC/MS to identify the anaerobic biodegradation by-products of DNOC. From these results (Table 11), it is postulated that under anaerobic conditions the DNOC underwent an initial breakdown that resulted in molecular structural changes, primarily in the loss of its nitro groups, with one of them substituted by a methyl group. The GC/MS results also showed that the breakdown of sucrose under anaerobic conditions resulted, as was expected, in the formation of organic

TABLE 9. REMOVAL OF DNOC IN THE ANAEROBIC COLUMN*

DNOC inf (mg/l)	DNOC eff (mg/l)	Removal of DNOC (%)
10	Not Detected	>99.9
20	N.D.	"
30	N.D.	"
50	N.D.	"
75	N.D.	"
100	N.D.	"
125	N.D.	"
150	N.D.	"
200	N.D.	"
250	N.D.	"
325	N.D.	"
400	N.D.	"
500	N.D.	"
600	N.D.	"

*Sucrose influent concentration = 3 g/l, hydraulic retention time = 3 days.

Table 10. THE EFFECT OF HYDRAULIC RETENTION TIME (HRT) ON THE ANAEROBIC COLUMN PERFORMANCE*

HRT (days)	DNOC_{inf} (mg/l)	DNOC_{eff} (mg/l)	DNOC Removal (%)
3.0	250	0.48	99.8
	"	Not Detected	>99.9
2.5	"	0.64	99.7
	"	0.54	99.8
2.0	"	0.25	99.9
	"	0.25	99.9
1.6	"	0.21	>99.9
	"	0.54	99.8
1.2	"	N.D.	>99.9
	"	N.D.	>99.9
0.93	"	N.D.	>99.9
	"	N.D.	>99.9
0.72	"	0.01	>99.9
	"	0.60	99.8
	"	0.12	>99.9
0.58	"	0.08	>99.9
	"	0.05	>99.9
	"	0.14	>99.9
0.43	"	N.D.	>99.9
	"	0.12	>99.9
	"	0.09	>99.9
0.28	"	0.02	>99.9
	"	0.03	>99.9
0.17	"	0.06	>99.9
	"	N.D.	>99.9

*Influent sucrose concentration = 2.0 g/l.

TABLE 11. BY-PRODUCTS OF THE DEGRADATION OF DNOC AND SUCROSE AS IDENTIFIED BY GC/MS[1,2]

Compound	Sample (acid extracted)				
	Anaerobic shaker bottle batch test	Anaerobic continuously-stirred batch test	Anaerobic column effluent[3]		Activated sludge effluent
			I	II	
DNOC	X				
2-Methyl-5-(1-methylethane)-phenol	X	X			
Pentanoic acid	X	X	X	X	
Butanoic acid			X	X	
Acetic acid denvatives			X	X	
Some alkanes			X	X	
3,4-Dimethyl phenol			X		
1,1-Bicyclohexyl			X	X	X

1. GC/MS analysis performed by D. Kampbell at the U.S. E.P.A. RSKERL, Ada, OK.

2. Identification criteria: matching base peaks, matching mass spectra and fit of >900.

3. The two anaerobic column effluent samples were taken from 2 different runs.

acids: pentanoic and butanoic acids and an array of acetic acids derivatives. The subsequent aerobic biodegradation in the activated sludge process resulted in the elimination of most of the anaerobic degradation by-products that were present in the anaerobic column effluent. The GC analysis performed on grab samples of gas produced in the anaerobic column provided further information on the by-products of the anaerobic breakdown process. Gas analyses were performed when gas production exceeded 3 liters/day. Based on these limited data, the gas composition was 82 to 85% CO_2, 17 to 15% N_2 and less than 1% CH_4. These data indicate that acid-producing bacteria, rather than the more sensitive methane-producing bacteria, dominated the microorganisms population in the anaerobic column. The production of nitrogen gas also indicate that a denitrification process also occurred in the anaerobic column. Thus the denitrifying microorganisms are responsible for the reduction of the nitro groups that originated from the DNOC molecule, to nitrogen gas.

B) THE CO-METABOLISM OF DNOC IN ANAEROBIC CONDITIONS

Sucrose as a Co-substrate

The continuously-stirred batch test results indicated that the degradation of DNOC occurring under anaerobic conditions was a result of the co-metabolism of the nitroaromatic compound and depended upon the presence of a co-substrate. In the presence of the co-substrate sucrose, DNOC was anaerobically co-metabolized. As can be seen from Tables 6, 12, 13, and 14 there is a direct correlation between the concentration of sucrose and the removal of DNOC in the anaerobic batch tests. The results presented in Figure 5 and Table 6 demonstrated the phenomenon which is the essence of any co-metabolic process; namely, the inability of the microorganisms to utilize the DNOC as its sole source of carbon and energy. As observed from Figure 5, when there is no sucrose present, the concentration

TABLE 12. THE EFFECT OF SUCROSE CONCENTRATIONS ON THE CO-METABOLISM OF DNOC IN CONTINUOUSLY-STIRRED BATCH TEST, RUN 1*

Sample	Time (days)	pH	SS (mg/l)	VSS (mg/l)	CODs (mg/l)	TOC (mg/l)	DNOC (mg/l)	Sucrose (g/l)
M	0	8.0	35	13	5400	2880	250	4.50
	1	7.0	122	102	4940	2440	229	2.68
	2	7.0	144	110	5040	2010	168	2.04
	3	7.0	152	126	4470	2260	130	1.00
	4	6.9	197	173	4530	2120	57.8	0
	5	6.9	160	140	4500	1850	11.7	0
	6	6.9	168	140	4600	2290	0.4	0
	7	6.9	223	171	4680	2220	N.D.	0
N	0	7.9	35	8	2130	1010	250	1.50
	1	6.8	107	88	1910	950	230	0.52
	2	6.6	70	60	1890	810	215	0.20
	3	6.6	72	64	1800	810	206	0
	4	6.6	110	98	1850	890	201	0
	5	6.6	85	74	1820	800	193	0
	6	6.6	96	78	1840	880	186	0
	7	6.6	110	87	1870	890	183	0
O	0	8.1	41	10	6590	4090	250	6.00
	1	7.1	96	78	6560	3700	223	4.10
	2	7.0	68	52	6310	3310	171	3.60
	3	7.0	62	50	6120	3290	135	3.16
	4	6.9	115	97	5910	3250	83.8	0.20
	5	6.9	71	58	5840	3330	9.2	0.16
	6	6.9	67	48	5850	3440	0.3	0.02
	7	6.9	124	84	6080	3500	N.D.	0.02

* Initial concentrations:

Sample	DNOC (mg/l)	Sucrose (g/l)	$NaHCO_3$ (g/l)	$(NH_4)_2HPO_4$ (g/l)
M	250	4.5	11.25	1.875
N	250	1.5	3.75	0.625
O	250	6.0	13.75	1.875

TABLE 13. THE EFFECT OF SUCROSE CONCENTRATIONS ON THE CO-METABOLISM OF DNOC IN CONTINUOUSLY-STIRRED BATCH TEST, RUN 2*

Sample	Time (days)	pH	SS (mg/l)	VSS (mg/l)	CODs (mg/l)	TOC (mg/l)	DNOC (mg/l)	Sucrose (g/l)
P	0	8.1	110	52	5620	2670	500	4.50
	1	7.8	113	56	5610	2670	500	4.47
	2	7.3	109	62	5600	2610	500	3.96
	3	6.6	128	88	5280	2650	490	2.84
	4	6.4	154	118	5150	2390	413	1.71
	5	6.4	161	116	5200	2190	327	1.52
	6	6.4	152	115	5020	2260	275	1.45
	7	6.4	150	120	5100	2190	256	1.45
Q	0	8.2	108	58	7260	3130	500	6.00
	1	8.0	141	72	7210	3000	500	6.00
	2	6.9	101	70	7050	3010	474	4.62
	3	6.7	62	40	6790	2950	394	3.84
	4	6.5	80	58	6560	2810	308	2.07
	5	6.4	72	45	6600	2790	181	1.75
	6	6.4	66	42	6400	2680	69.6	1.46
	7	6.4	64	40	6400	2870	14.9	1.41
R	0	8.1	120	65	8780	3900	500	7.50
	1	7.9	154	84	8730	3600	500	7.50
	2	7.0	117	78	8580	3610	455	5.93
	3	6.8	68	46	8230	3500	387	5.14
	4	6.8	99	63	8470	3570	359	4.61
	5	6.7	107	74	8240	3440	272	3.51
	6	6.7	106	71	7940	3570	136	3.00
	7	6.7	99	68	7850	3450	42.0	2.57

* Initial concentrations:

Sample	DNOC (mg/l)	Sucrose (g/l)	$NaHCO_3$ (g/l)	$(NH_4)_2HPO_4$ (g/l)
P	500	4.5	5.0	1.25
Q	500	6.0	6.5	1.75
R	500	7.5	8.75	2.25

TABLE 14. THE EFFECT OF SUCROSE CONCENTRATIONS ON THE CO-METABOLISM OF DNOC IN CONTINUOUSLY-STIRRED BATCH TEST, RUN 3*

Sample	Time (days)	pH	SS (mg/l)	VSS (mg/l)	CODs (mg/l)	TOC (mg/l)	DNOC (mg/l)	Sucrose (g/l)
V	0	7.7	68	30	2480	1160	100	2.00
	1	6.1	170	138	2140	900	99.0	0.20
	2	6.1	156	116	2030	950	80.4	0.16
	3	6.1	161	134	2080	930	74.6	0.16
	4	6.1	162	137	2070	890	64.3	0.16
	5	6.1	159	133	2070	923	58.0	0.16
	6	6.1	166	133	2100	917	56.5	0
	7	6.1	158	134	2080	927	52.9	0
W	0	7.8	66	30	1360	695	100	1.00
	1	6.6	113	81	1170	608	100	0.23
	2	6.6	109	78	1100	663	91.6	0.14
	3	6.6	121	88	1090	616	87.9	0
	4	6.6	120	89	1070	633	79.3	0
	5	6.6	120	91	1050	618	66.2	0
	6	6.6	116	88	1050	644	62.6	0
	7	6.6	128	97	1020	688	60.6	0
X	0	7.9	70	20	847	512	100	0.50
	1	6.9	115	70	732	522	100	0.28
	2	6.9	97	56	676	517	100	0.16
	3	6.9	98	61	652	530	100	0
	4	6.9	97	63	636	541	96.5	0
	5	6.9	94	55	610	497	93.3	0
	6	6.9	103	69	597	591	89.9	0
	7	6.9	97	64	594	575	88.1	0

* Initial concentrations:

Sample	DNOC (mg/l)	Sucrose (g/l)	$NaHCO_3$ (g/l)	$(NH_4)_2HPO_4$ (g/l)
V	100	2.0	3.5	0.5
W	100	1.0	3.5	0.5
X	100	0.5	3.5	0.5

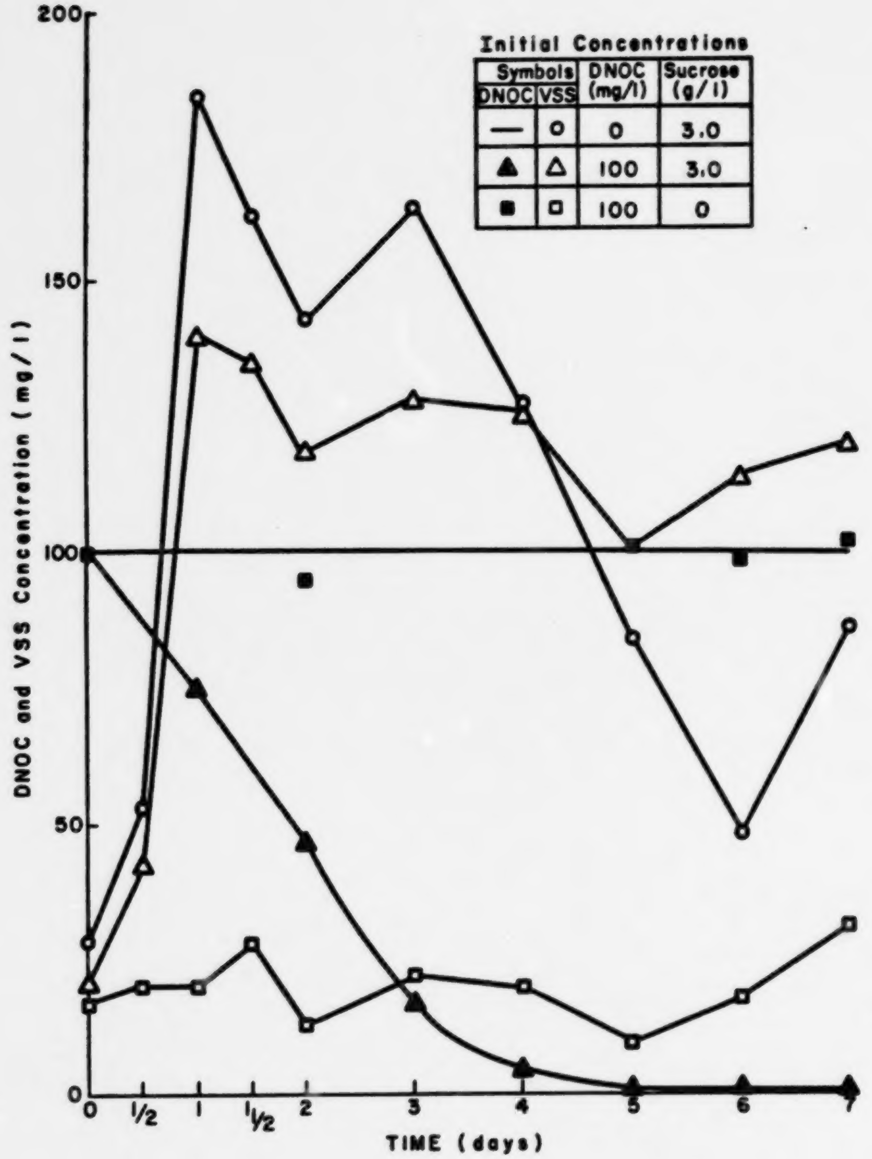


Figure 5. The effect of sucrose concentration on the removal of DNOC in continuously-stirred batch test.

of DNOC remained unchanged. This also resulted in a drastic inhibition of biomass growth; i.e. no change in VSS concentration was observed over time, showing that the DNOC itself is toxic to the anaerobic microorganisms when a readily biodegradable co-substrate is not available.

The Effect of Sucrose and DNOC Concentrations on the Performance of the Anaerobic Column in the Co-metabolism of DNOC

Early results from anaerobic continuously-stirred batch tests (Tables 15 and 16) showed that the anaerobic process failed to completely remove DNOC over a period of 7 days when the DNOC initial concentrations exceeded 200 mg/l and the sucrose concentration was maintained at 3.0 g/l. These data indicated that the ratio of sucrose to DNOC concentrations might be an important parameter controlling the efficiency of the anaerobic co-metabolism of DNOC. The removal of DNOC in the anaerobic column was therefore studied under the following conditions:

I. Influent DNOC concentration = 250 mg/l
Influent sucrose concentration = gradually reduced from
2.0 g/l to 0.25 g/l, HRT = 0.47 days.

The results of these studies are shown in Figure 6.

II. Influent DNOC concentration = 500 mg/l
Influent sucrose concentration = gradually reduced from
3.0 g/l to 0.75 g/l, HRT = 3.0 days.

The results of these studies are shown in Figure 7.

The performance of the anaerobic column in the removal of DNOC was highly dependent on the influent concentration of the co-substrate, sucrose. A ratio of influent sucrose to DNOC of 2:1 or higher resulted in 95-100% removal (or conversion) of DNOC in the anaerobic process. However, when influent sucrose to DNOC ratio was less than 2:1 the anaerobic microorganisms failed to co-metabolize DNOC.

TABLE 15. THE EFFECT OF DNOC CONCENTRATIONS ON THE CO-METABOLISM OF DNOC IN CONTINUOUSLY-STIRRED BATCH TEST*

Sample	Time (days)	pH	SS (mg/1)	VSS (mg/1)	TOA (mg/1)	COD_s (mg/1)	TOC (mg/1)	DNOC (mg/1)	Sucrose (g/1)
F	0	7.8	48	22	--	3500	1620	50.0	3.0
	1	7.0	151	130	2630	3140	1380	19.3	0
	2	7.0	122	93	--	3240	1360	1.4	0
	3	6.9	116	92	2450	3030	1390	N.D.	0
	4	6.9	116	100	--	3040	1300	--	0
	5	6.9	96	82	--	3080	1500	--	0
	6	6.9	106	86	--	3140	1580	--	0
	7	6.9	146	122	--	3150	1600	--	0
G	0	7.9	34	15	--	3630	1720	150	3.0
	1	7.0	144	119	2710	3290	1390	51.4	0
	2	6.9	103	78	--	3350	1420	15.0	0
	3	6.9	90	71	2530	3140	1380	1.0	0
	4	6.9	110	90	--	3170	1490	0.3	0
	5	6.9	106	89	--	3210	1490	0.1	0
	6	6.9	110	93	--	3260	1680	N.D.	0
	7	6.9	111	91	--	3290	1690	N.D.	0
H	0	7.8	21	6.5	--	3680	1660	200	3.0
	1	7.1	128	98	2010	3390	1520	84.0	0
	2	7.0	123	94	--	3390	1540	26.4	0
	3	6.9	113	92	2500	3110	1340	0.6	0
	4	6.9	122	105	--	3160	1410	0.3	0
	5	6.9	114	97	--	3160	1480	0.2	0
	6	6.9	137	124	--	3240	1590	N.D.	0
	7	6.9	151	127	--	3360	1750	N.D.	0

*Initial Concentrations:

Sample	DNOC (mg/1)	Sucrose (g/1)	$NaHCO_3$ (g/1)	$(NH_4)_2HPO_4$ (g/1)
F	50	3.0	10.7	1.25
G	150	3.0	10.7	1.25
H	200	3.0	10.7	1.25

TABLE 16. THE EFFECT OF HIGH DNOC CONCENTRATIONS ON THE CO-METABOLISM OF DNOC IN CONTINUOUSLY-STIRRED BATCH TEST*

Sample	Time (days)	pH	SS (mg/1)	VSS (mg/1)	COD_s (mg/1)	TOC (mg/1)	DNOC (mg/1)	Sucrose (g/1)
J	0	8.0	35	21	3730	1850	250	3.00
	1	6.8	132	102	3450	1400	201	0.88
	2	6.8	102	86	3400	1320	152	0.285
	3	6.8	73	57	3330	1350	115	0
	4	6.8	65	52	3410	1400	108	0
	5	6.8	57	50	3450	1620	100	0
	6	6.8	51	47	3470	1700	94.5	0
	7	6.8	59	49	3370	1530	83.0	0
K	0	7.9	37	20	4040	1520	500	3.00
	1	7.5	64	32	4000	1680	500	2.57
	2	7.3	62	35	3940	1600	493	2.40
	3	6.9	43	32	3660	1530	470	0.67
	4	6.9	32	26	3720	1360	390	0.65
	5	6.9	34	30	3740	1660	311	0.59
	6	6.9	41	34	3790	1660	298	0.56
	7	6.9	62	51	3690	1590	253	0.49
L	0	8.0	32	15	4290	1560	750	3.00
	1	7.9	58	25	4350	1600	750	3.00
	2	7.8	45	19	4300	1690	750	3.00
	3	7.7	48	20	4180	1660	750	2.96
	4	7.7	54	34	4300	1600	750	2.93
	5	7.5	54	33	4260	2020	739	2.90
	6	7.5	48	25	4300	1940	726	2.88
	7	7.4	56	34	4140	1920	712	2.79

*Initial concentrations:

Sample	DNOC (mg/1)	Sucrose (g/1)	$NaHCO_3$ (g/1)	$(NH_4)_2HPO_4$ (g/1)
J	250	3.0	10.0	1.25
K	500	3.0	10.0	1.25
L	750	3.0	10.0	1.25

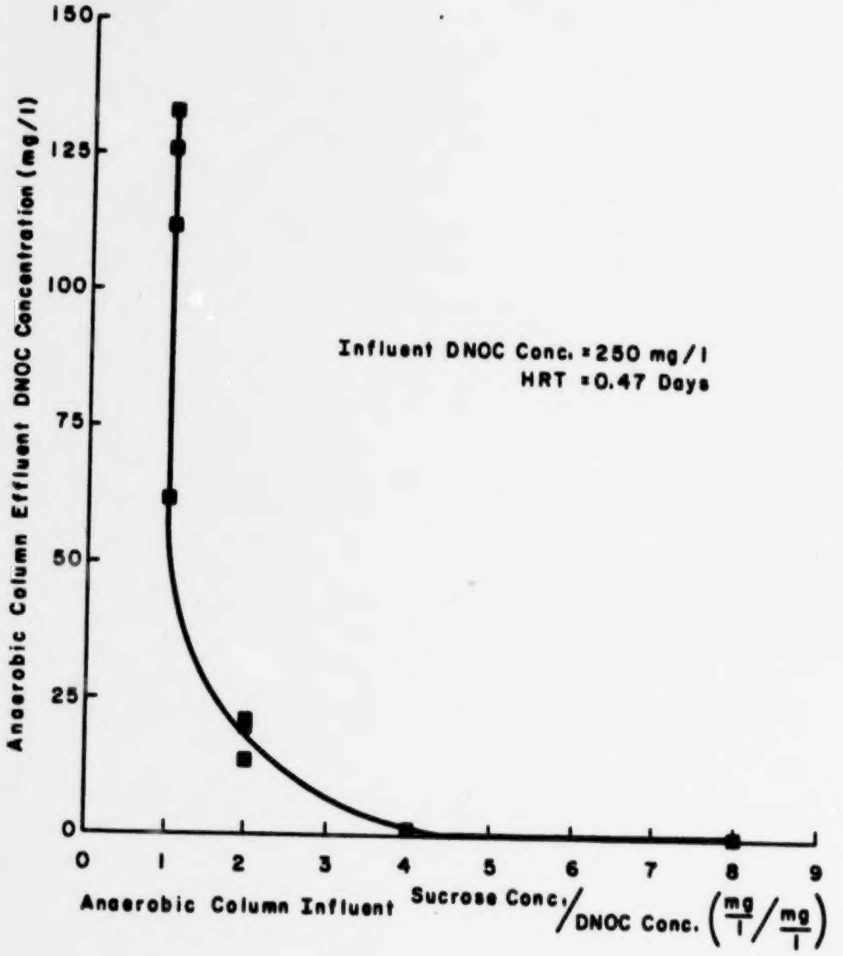


Figure 6. The effect of sucrose/DNOC concentration ratio on the removal of DNOC in the anaerobic column, run 1.

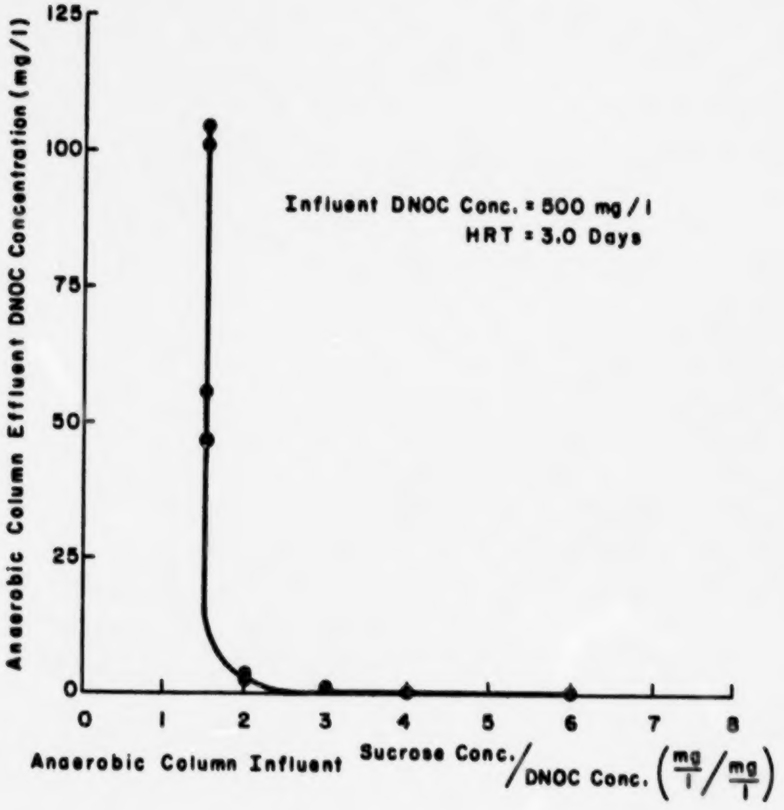


Figure 7. The effect of sucrose/DNOC concentration ratio on the removal of DNOC in the anaerobic column, run 2.

C) THE REMOVAL OF COD IN THE ANAEROBIC-AEROBIC SYSTEM

It has been established that DNOC will be degraded or converted under anaerobic conditions in the presence of sucrose as a co-substrate. The degradation of the DNOC molecule in the anaerobic process, monitored by GC, was not associated with an appreciable decrease in soluble COD. Early batch tests indicated in one case (Table 4) that over a period of 7 days a DNOC removal of 85% was associated with 16% decrease in soluble COD, which was coupled with a 500% increase in TOA, or in another case (Table 6) that over the same period of time a removal of 99.8% of DNOC was associated with only an 11% decrease in soluble COD, while the TOA level increased by 700%. These early data demonstrated that in the presence of sucrose, the anaerobic microorganisms co-metabolized the DNOC molecule and, thereby, caused some initial structural change(s) in or around its benzene nucleus. However, since no appreciable COD reduction occurred in the anaerobic process and a very large increase in TOA occurred, the two main processes which took place in the anaerobic system were: a) the co-substrate, sucrose, was degraded by the anaerobic bacteria to organic acids resulting in a large increase in TOA concentration and an insignificant decrease in soluble COD and b) the structural conversion of the DNOC molecule via co-metabolism by the anaerobic bacteria which was dependent on the presence of the co-substrate sucrose.

The reduction in COD levels across the entire anaerobic-aerobic pilot plant was studied under varied conditions of hydraulic retention time in the anaerobic column and varied influent sucrose concentrations.

The Effect of Hydraulic Retention Time on the Removal of Soluble COD

The effect of the hydraulic retention time (HRT) in the anaerobic column on the removal of soluble COD is shown in Table 17. Influent sucrose and DNOC concentrations were maintained at 2.0 g/l and 250 mg/l,

TABLE 17. THE EFFECT OF ANAEROBIC COLUMN HYDRAULIC RETENTION TIME (HRT) ON SOLUBLE COD REMOVAL BY THE ANAEROBIC-AEROBIC SYSTEM

HRT (days)	DNOC removed (%)	COD Removal in AC (%)	COD Removal in AS (%)	COD Removal in Total (%)
0.16	>>99.9	26.1	24.0	50.1
0.28	99.9	20.5	49.2	69.7
0.43	99.9	24.6	66.6	91.2
0.72	99.9	24.9	65.9	90.8
1.62	99.8	21.7	69.6	91.3
2.0	99.9	20.5	68.6	89.1
2.5	99.8	15.7	76.8	92.5
3.0	99.8	3.2	86.7	89.9

Influent sucrose concentration = 2.0 g/l
Influent DNOC concentration = 250 mg/l

respectively. The HRT in the anaerobic column was varied from 0.16 days to 3.0 days and the soluble COD removal in the anaerobic column varied from 26% to 3%, respectively. The soluble COD removal achieved in the subsequent activated sludge reactor varied from 24% to 87% resulting in an overall soluble COD removal of 50% to 90%, respectively. The results demonstrated that within the operational conditions of the system, a short HRT in the anaerobic column such as 0.16 days and 0.28 days had an adverse effect on the overall removal of COD, 50% and 70% removal respectively, while a HRT of 0.43 days and longer resulted in overall COD removals of about 90%.

The Effect of Influent Sucrose Concentration on the Removal of Soluble COD

The effect of influent sucrose concentration on the removal of soluble COD through the entire anaerobic-aerobic system was studied under two sets of conditions:

I. For the first set of conditions, the effect of influent sucrose concentration was monitored at moderate influent DNOC concentration and low HRT in the anaerobic column. The influent DNOC concentration was maintained at 250 mg/l, the HRT in the anaerobic column was maintained at 0.47 days and influent sucrose concentration varied from 0.25 g/l to 2.0 g/l.

II. For the second set of conditions, the effect of influent sucrose concentration was monitored at relatively high influent DNOC concentration and high HRT in the anaerobic column. The influent DNOC concentration was maintained at 500 mg/l, the HRT in the anaerobic column was maintained at 3.0 days and influent sucrose concentration varied from 0.75 g/l to 3.0 g/l. The results for both sets of conditions appear in Table 18.

When the ratio of influent sucrose concentration to influent DNOC concentration was maintained at 2:1 or higher, the degradation (or conversion) of DNOC in the anaerobic column was between 92% and 100%. However, even when provided with adequate supply of co-substrate, sucrose

TABLE 18. THE EFFECT OF INFLUENT SUCROSE CONCENTRATION ON SOLUBLE COD REMOVAL BY THE ANAEROBIC-AEROBIC SYSTEM

			CODs removal in		
Set no.	AC_{inf} sucrose conc. (g/l)	DNOC removed (%)	AC (%)	AS (%)	Total (%)
1*	2.00	>>99.9	23.3	61.2	84.5
	1.00	99.4	14.7	71.3	86.0
	0.50	91.6	16.8	54.3	71.1
	0.25	50.4	10.3	42.5**	52.8**
2***	3.0	>>99.9	27.3	63.2	90.5
	3.0	>>99.9	30.0	58.7	88.7
	2.0	>>99.9	26.4	58.7	85.1
	1.5	99.6	21.8	56.9	78.7
	1.5	99.9	30.1	52.3	82.4
	1.0	99.6	30.8	48.4	79.2
	0.75	88.9	24.0	44.6**	68.6**
	0.75	79.8	19.5	46.6**	66.1**

* Influent DNOC concentration = 250 mg/l
HRT = 0.47 days

** The activated sludge removal efficiency was significantly affected by the high concentrations of DNOC in the anaerobic column effluent.

*** Influent DNOC concentration - 500 mg/l
HRT = 3.0 days

to achieve more than 90% conversion of DNOC, the anaerobic microorganisms were unable to decrease the soluble COD by more than 23% in the first set of conditions and 30% in the second set of conditions. The importance of the subsequent aerobic stage in the removal of the remaining high COD is clearly demonstrated. The soluble COD removal that was achieved in the activated sludge process (following an anaerobic DNOC conversion of 90% or higher) ranged from 54 to 71% under the first set of conditions and from 48 to 63% under the second set of conditions.

It is also important to note that the performance of the activated sludge process was adversely affected when the ratio of sucrose:DNOC in the anaerobic column influent was less than 2:1 as shown in Figures 8 and 9. This resulted in failure of the anaerobic microorganisms to satisfactorily co-metabolize DNOC, and, subsequently, high concentrations of DNOC were present in the anaerobic column effluent and entered into the activated sludge reactor.

D) CHANGES IN MICROTOX^{TM} TOXICITY THAT WERE ASSOCIATED WITH THE REMOVAL OF DNOC IN THE ANAEROBIC-AEROBIC SYSTEM.

The effect of influent sucrose concentration on Microtox^{TM} toxicity through the anaerobic-aerobic system was studied under the following set of conditions: the influent DNOC concentration was maintained at 250 mg/l, the HRT in the anaerobic column was maintained at 0.47 days and the influent sucrose concentration was varied from 2.0 g/l to 0.25 g/l.

As can be seen from Table 19, when comparing the Microtox^{TM} toxicities of the anaerobic column influent, the anaerobic column effluent and the activated sludge effluent, the anaerobic column effluent was more toxic to the Microtox^{TM} microorganisms than the anaerobic column influent; however, the activated sludge effluent was less toxic to the Microtox^{TM} bacteria than the anaerobic column effluent was suggesting that the aerobic biode-

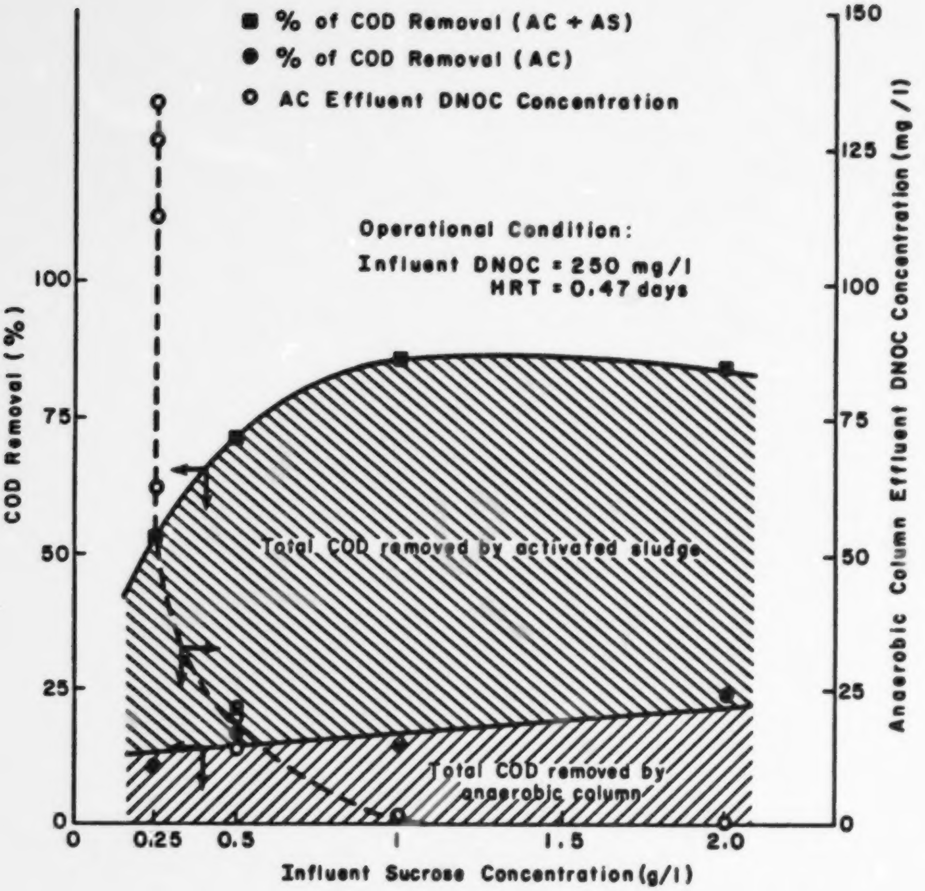


Figure 8. The effect of influent sucrose concentration on the removal of COD by anaerobic column and activated sludge, run 1.

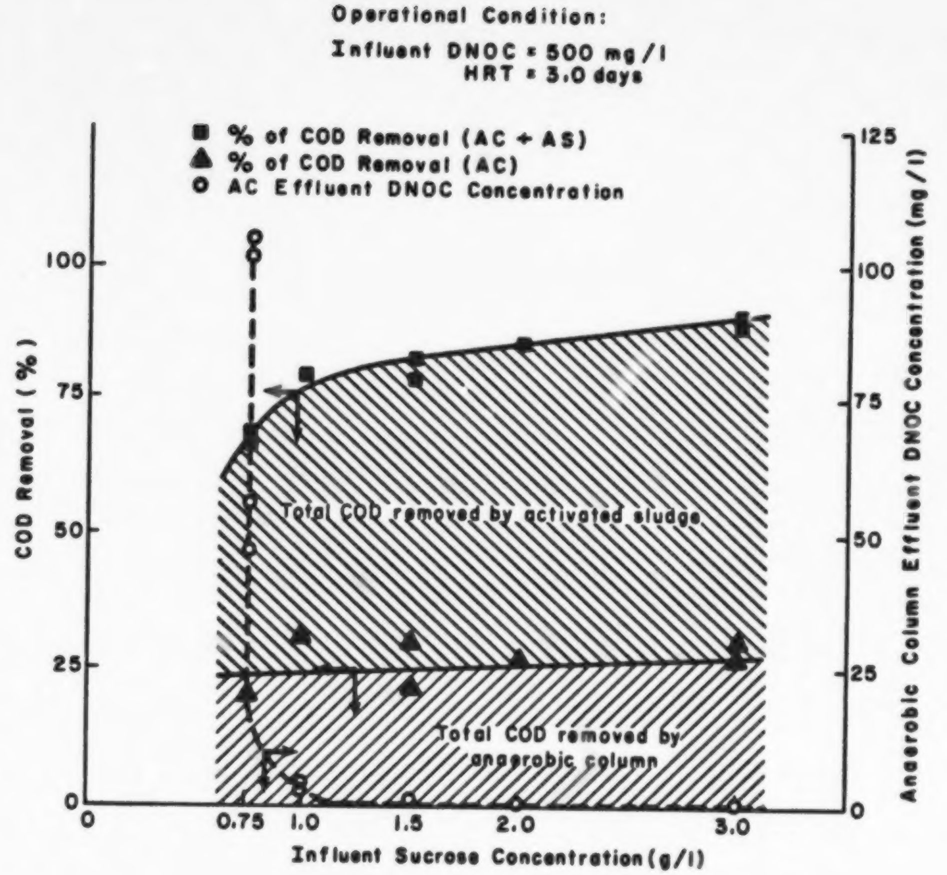


Figure 9. The effect of influent sucrose concentration on the removal of COD by anaerobic column and activated sludge, run 2.

gradation that occurred in the activated sludge process was principally responsible for the observed reduction in Microtox^{TM} toxicity. However, from the results presented in Tables 19 and 20 it is also clear that the Microtox^{TM} toxicities, that were demonstrated by the anaerobic-column effluent and the activated sludge effluent were mainly a result of the anaerobic degradation of the sucrose and the build up of organic acids in the anaerobic column. These organic acids were subsequently further degraded to CO_2 and H_2O in the activated sludge process, when the hydraulic retention time in the system was long enough and the initial sucrose:DNOC ratio was 2:1 or higher. Since DNOC did not undergo biodegradation in the absence of the co-substrate sucrose, it was impossible to monitor the Microtox^{TM} toxicity changes that were associated with the biodegradation of DNOC only, without interference of the toxicity effects resulting from the degradation of sucrose to organic acids in the anaerobic column. Accordingly, no conclusions were drawn about the changes in DNOC toxicity from the Microtox^{TM} data.

TABLE 19. THE EFFECT OF INFLUENT SUCROSE CONCENTRATION ON MICROTOX^{TM} TOXICITY THROUGH THE ANAEROBIC-AEROBIC SYSTEM

Influent DNOC (mg/l)	Influent Sucrose (g/l)	HRT in AC (days)	DNOC removal in AC (%)	AC influent EC_{50}*(5)	AC influent EC_{50}(15)	AC effluent EC_{50}(5)	AC effluent EC_{50}(15)	AS effluent EC_{50}(5)	AS effluent EC_{50}(15)
250	2.0	0.47	>>99.9	19.36	13.84	0.673	0.44	9.97	8.48
250	1.0	0.47	99.4	7.34	6.18	0.865	0.62	10.25	8.27
250	0.5	0.47	91.6	10.77	9.74	5.2	5.3	5.73	5.55
250	0.25	0.47	49.6	10.92	8.90	5.95	5.22	3.63	3.52
0	2.0	0.47	-	63.16	51.57	4.3×10^{-5}	7.9×10^{-2}	2.78	2.28

* EC_{50} is expressed in units of % of initial sample concentration.

TABLE 20. MICROTOX^{TM} TOXICITY DATA FOR THE ANAEROBIC-AEROBIC SYSTEM.

Influent		HRT	AC influent		AC effluent		AS effluent	
DNOC (mg/l)	Sucrose (g/l)	in AC (days)	EC_{50}*(5)	EC_{50}(15)	EC_{50}(5)	EC_{50}(15)	EC_{50}(5)	EC_{50}(15)
750	2.0	3.0	5.69 6.44	5.37 4.72	2.26	2.21	4.75	4.49
500	1.5	3.0	9.60	6.52			2.12	2.03
500	1.5	3.0	6.83	4.62	2.45	2.60	2.12	2.03
250	2.0	0.43	19.4	13.8	0.67	0.43	13.1	9.18
250	2.0	0.28	19.4	13.8	0.26	<0.1		
250	2.0	0.7	19.36	13.84	0.707	0.40	7.53	10.6
50	2.0	0.28	14.7	13.1	0.69	0.52	16.5	14.0

* EC_{50} is expressed in units of % of initial sample concentration.

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APPENDIX A

EXPERIMENTAL APPARATUSES AND PROCEDURES

ANAEROBIC SHAKER BOTTLE BATCH TEST APPARATUS AND PROCEDURES

Apparatus

a) Glass bottles with screw caps, each with 240 ml capacity.

b) Shaker water bath with temperature control.

Procedures

a) Each batch test lasted seven days with a single DNOC concentration in each batch test run. A total of eight bottles were needed for each run to supply eight incubation time samples, each one taken once every 24 hours, for incubation duration periods of 0 to 7 days.

b) Purge the bottles with oxygen-free nitrogen gas.

c) Add in 170 ml of DNOC-feed solution. For composition of DNOC-feed solution see Table A-1.

DNOC is added into the feed solution from an aqueous alkaline stock solution of 10,000 mg/l or 20,000 mg/l DNOC, to make the desired final DNOC concentration with a total volume of 200 ml.

d) Mix the solution in the bottles with nitrogen gas.

e) Collect a sufficient amount of anaerobic upflow column, mix with oxygen-free nitrogen gas to ensure homogeneous distribution of its content.

f) Carefully transfer 30 ml of anaerobic effluent into each bottle to serve as seed. Each bottle contains now a total volume of 200 ml.

g) After inoculation quickly purge the bottle top space with nitrogen gas and immediately seal the top of the bottle with a sheet of parafilm^{R}, then screw the cap tight.

h) Use one of the eight bottles as time 0 sample, subject it to

TABLE A-1. COMPOSITION OF DNOC-FEED SOLUTION

Compound	Concentration (mg/l)
DNOC	varies
sucrose	varies
$NaHCO_3$	varies*
$(NH_4)_2HPO_4$	varies**
minerals:	
$FeCl_3 \cdot 6H_2O$	10
$CaCl_2 \cdot 6H_2O$	5
$MgCl_2 \cdot 6H_2O$	5
$NiCl_2 \cdot 6H_2O$	5
$ZnCl_2$	5
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	1.5
$CuCl_2$	0.5
$MnCl_2 \cdot 4H_2O$	0.5
$Na_2B_4O_7 \cdot H_2O$	0.5
dilution water	dechlorinated tap water

*Concentration of $NaHCO_3$ added is adjusted to maintain desired pH.
**Concentration of $(NH_4)_2HPO_4$ added is adjusted to maintain COD:N=100:5.

analyses.

i) Put the remaining seven bottles in the 36.5°C water bath. Turn on the shaker and adjust the shaking speed to 100 strokes per minute.

j) Stop the incubation of one bottle every 24 hours by removing it from the water bath and subject it to analyses, continue incubation of remaining bottles with the last bottle removed at the end of the seventh day.

k) Right after the daily sample is taken, it is analyzed for pH, oxidation-reduction potential (ORP), suspended solids (SS), volatile suspended solids (VSS), and toxicity. Samples for soluble chemical oxygen demand (COD), soluble total organic carbon (TOC), and total organic acids (TOA) are also prepared. A sample is extracted and then analyzed by gas chromatography (GC) for determination of DNOC concentration. When necessary, an additional sample is hydrolysed and analyzed for residual sucrose concentration. Details of analytical methods are in Appendix C.

l) Note: during the first few days of the incubation period there is considerable gas production. It is, therefore, important to relieve the gas and pressure produced by slightly opening the bottles and closing them tight again every day.

ANAEROBIC CONTINUOUSLY-STIRRED BATCH TEST APPARATUS AND PROCEDURES

Apparatus

a) Three amber glass bottles with 4.2 liters capacity, each fitted with a three-hole rubber stopper, serve as batch-test bottles.

b) Heat tapes wrapped around the amber glass bottles.

c) Powerstat autotransformers to manually adjust the voltage applied to the heat tapes and maintain the temperature in the bottles at 36.5°C 1°C.

d) Heavy duty magnetic stirrer and a large stir bar for each batch test bottle.

e) Three additional bottles containing gas displacement fluid (aqueous

Na_2SO_4-H_2SO_4 solution prepared following Method 511A, Standard Methods (53)) each filled with a two-hole rubber stopper.

f) Container to collect displaced fluid from e.

g) Three thermometers, each one fitted into the rubber stopper of each of the three batch test bottles.

h) Tubing and clamps.

i) Apparatus set-up is shown in Figure A-1.

Procedures

a) To each batch test bottle add 3.75 liter of dilution water (tap water), minus the volume for DNOC stock solution.

b) Turn on the autotransformers and the magnetic stirrers, equilibrate to $36.5^{o}C \pm 1^{o}C$. Adjust the autotransformers if necessary.

c) Purge the batch test bottles as well as the bottles containing the gas displacement fluid with oxygen-free nitrogen gas.

d) For each batch test bottle add in the desired amounts of sucrose, $NaHCO_3$, $(NH_4)_2HPO_4$ and trace minerals to give the final concentrations as listed in Table A-1.

e) Add in DNOC stock solution (10,000 mg/l or 20,000 mg/l, in aqueous alkaline solution) to make the desired DNOC concentration with total volume of 4 liters.

f) Collect enough volume of anaerobic effluent from anaerobic upflow column, mix with oxygen-free nitrogen gas during collection time.

g) Carefully transfer 250 ml of anaerobic effluent into each batch test bottle for inoculation.

h) After inoculation, quickly purge the top space of the batch-test bottles with nitrogen gas and stop the bottles with the rubber stoppers.

i) Use parafilm^{R} sheet to build up the lip of the bottles and add water to ensure a water seal.

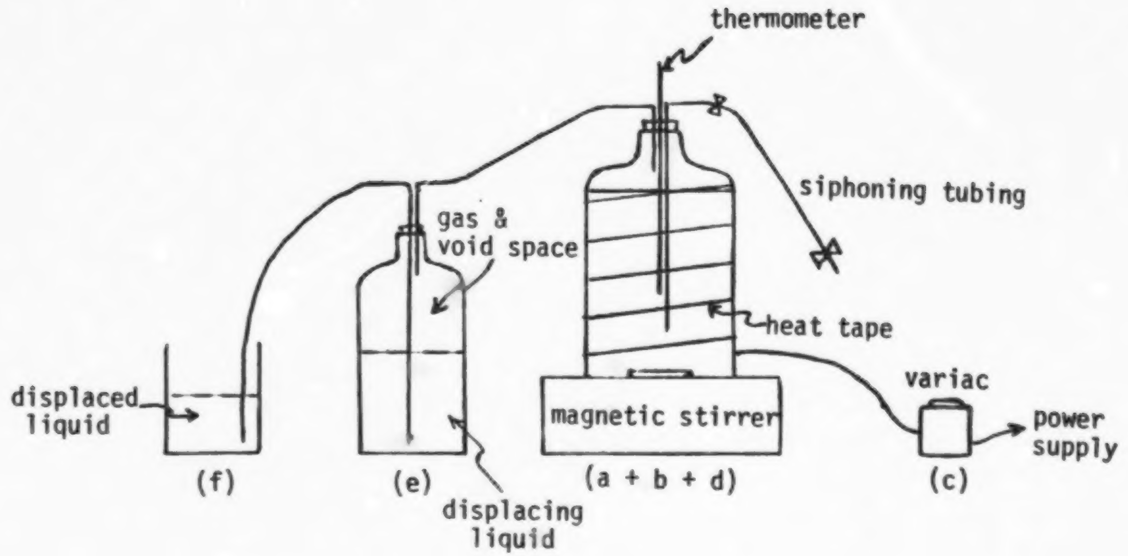
thermometer
siphoning tubing
gas &
void space
heat tape
variac
displaced
liquid
magnetic stirrer
power
supply
(f)
(e)
(a + b + d)
(c)
displacing
liquid

Figure A-1. Schematic diagram of continuously-stirred batch test apparatus.

j) Withdraw 250 ml immediately after inoculation, to serve as time 0 sample, by opening the clamp and siphoning with a section of tubing filled with water.

l) Withdraw 250 ml sample once every 24 hours for the following seven days.

m) Right after the daily sample is taken, it is analyzed for pH, oxidation-reduction potential (ORP), suspended solids (SS), volatile suspended solids (VSS), and toxicity. Samples for soluble chemical oxygen demand (COD), soluble total organic carbon (TOC), and total organic acids (TOA) are also prepared. A sample is extracted and then analyzed by gas chromatography (GC) for determination of DNOC concentration. When necessary, an additional sample is hydrolysed and analyzed for residual sucrose concentration. Details of analytical methods are in Appendix E.

n) Note: the withdrawal of the samples from the batch test bottles will cause the gas displacement fluid to flow from container (f), back to the gas collecting bottles (e). To prevent air from getting into the system at the time when a sample is withdrawn, the end of the tubing in container (f) should be well submerged at all times.

PILOT PLANT PROCEDURES

a) The anaerobic upflow sand filter column was started up by several inoculations with seed from a sludge digester of a local municipal sewage treatment plant. Operating parameters such as organic loadings, hydraulic retention times, loading concentrations of DNOC and of sucrose were carefully monitored and were changed when changes in operating parameters were called for by the protocol of a particular experiment, which was usually no less than three hydraulic retention time periods under previous operating conditions. These changes were introduced only when the column was operating under steady-state conditions.

b) The performance of the anaerobic column in the pretreatment process of DNOC was monitored by sampling its effluent and analyzing it for pH, alkalinity, oxidation-reduction potential (ORP), suspended solids (SS), volatile suspended solids (VSS), and toxicity. Samples for soluble chemical oxygen demand (COD) and soluble total organic carbon (TOC) were also prepared. A sample was extracted and then analyzed by gas chromatography (GC) for determination of DNOC concentration. When necessary, an additional sample was hydrolyzed and analyzed for residual sucrose concentration. When gas production in the column was significant, the volume of the off-gas produced was analyzed and its volume was measured. Details of analytical methods used are in Appendix C.

c) The performance of the activated sludge process in the treatment of the anaerobic-degradation-products was monitored by sampling its effluent and analyzing it for pH, COD, TOC, and toxicity.

APPENDIX B

ANAEROBIC CONTINUOUSLY-STIRRED BATCH TEST RESULTS

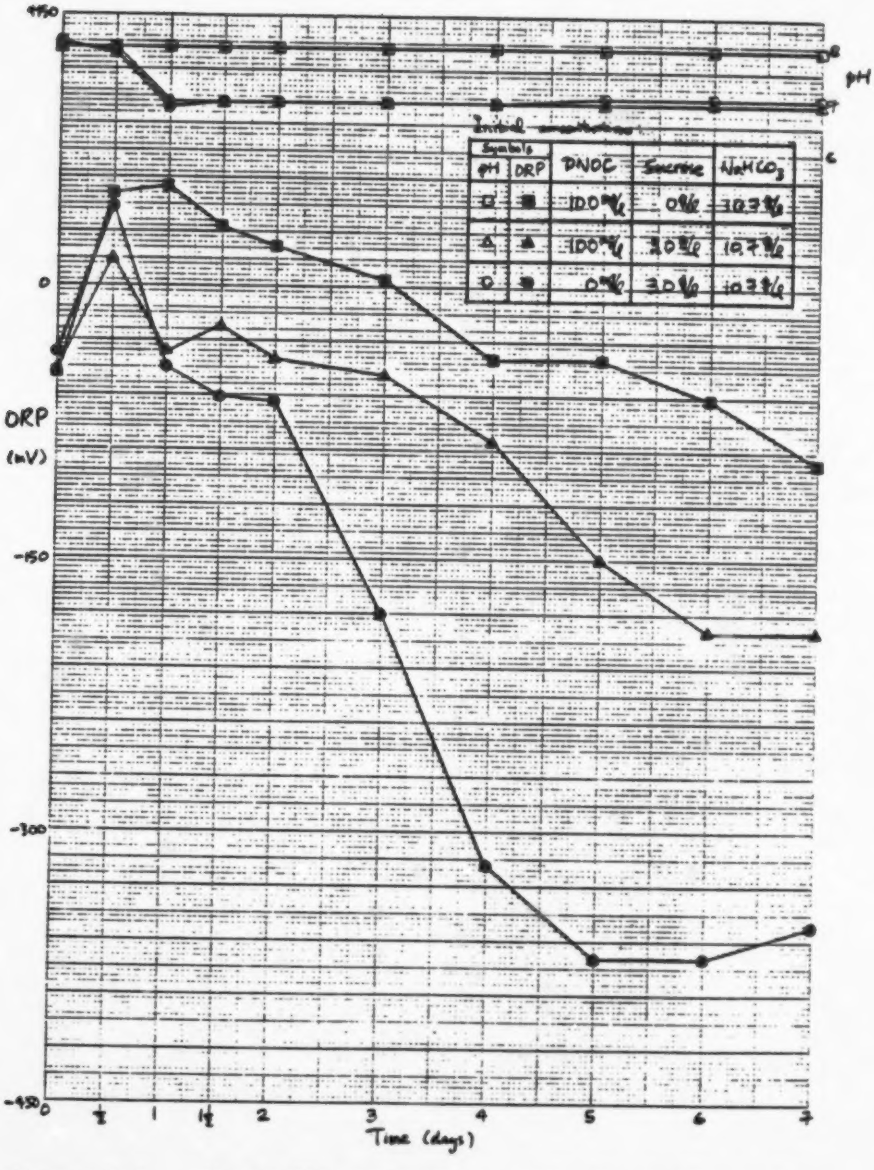

Figure B-1. pH and ORP changes vs. time in continuously-stirred batch test under the given initial concentrations.

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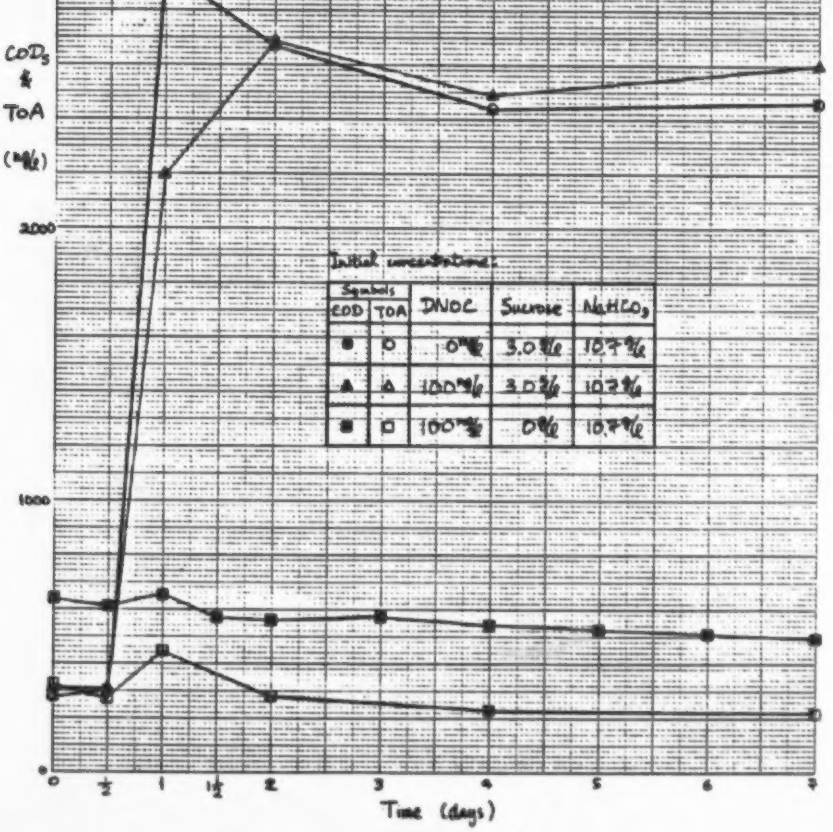

Figure B-2. COD and TOA changes vs. time in continuously-stirred batch test under the given initial concentrations.

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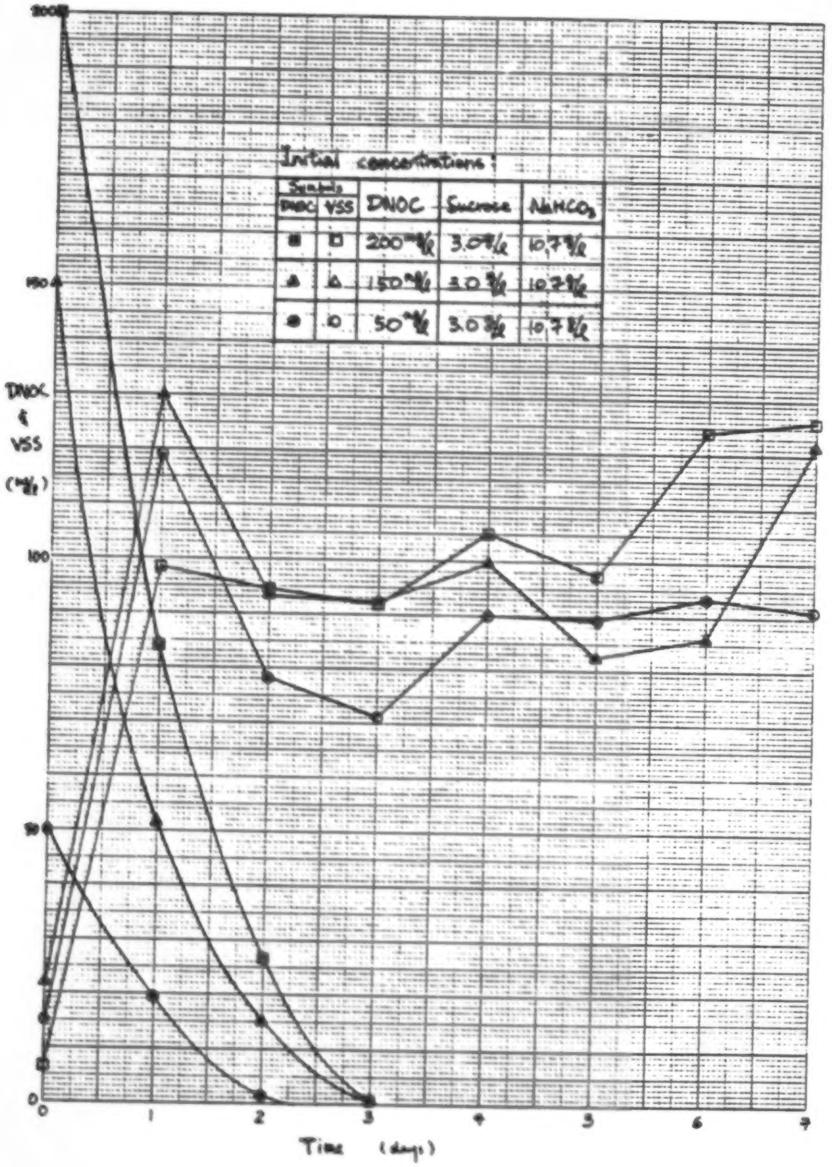

Figure B-3. DNOC and VSS changes vs. time in continuously-stirred batch test under the given initial concentrations.

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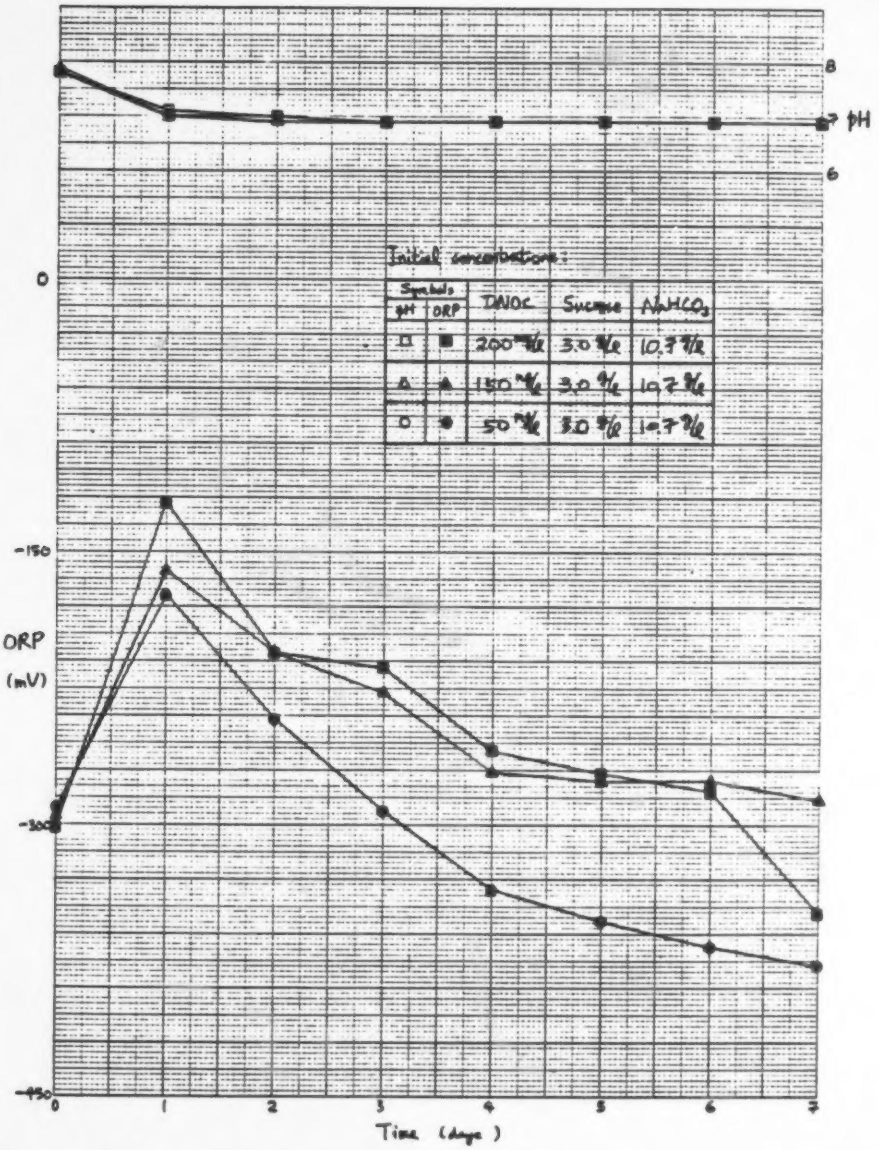

Figure B-4. pH and ORP changes vs. time in continuously-stirred batch test under the given initial concentrations.

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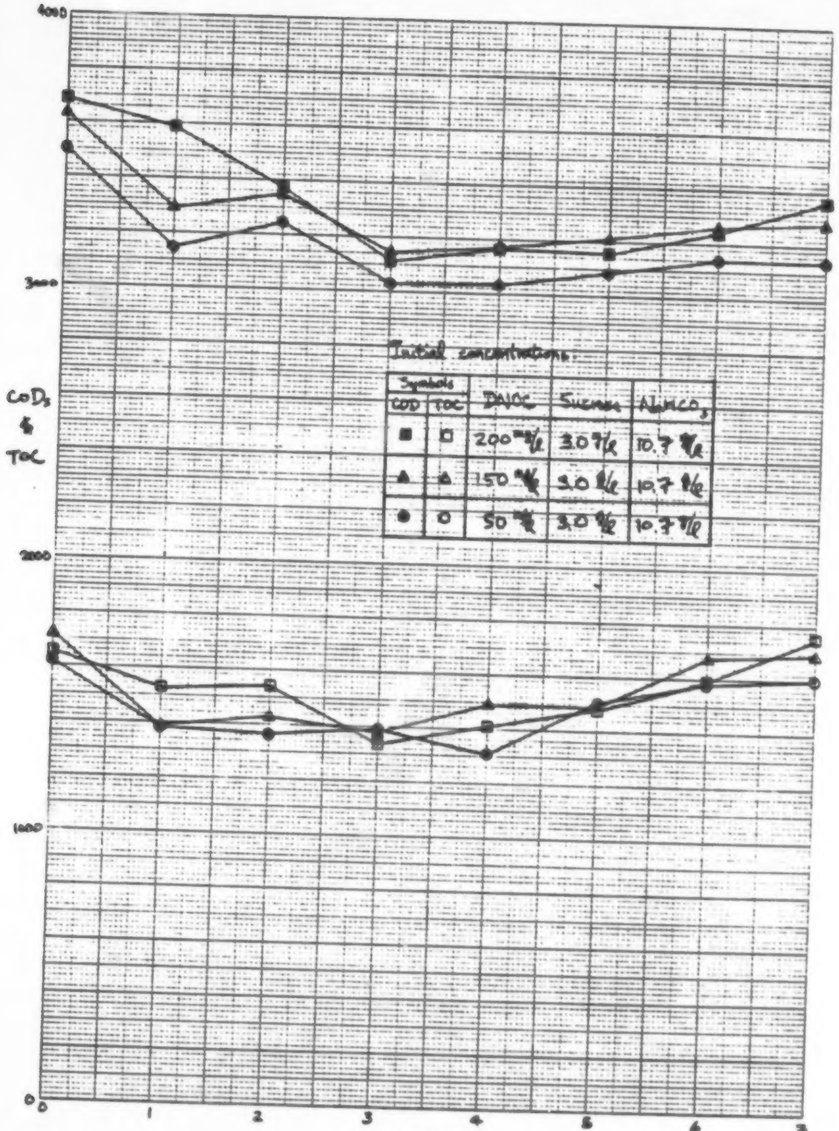

Figure B-5. COD and TOC changes vs. time in continuously-stirred batch test under the given initial concentrations.

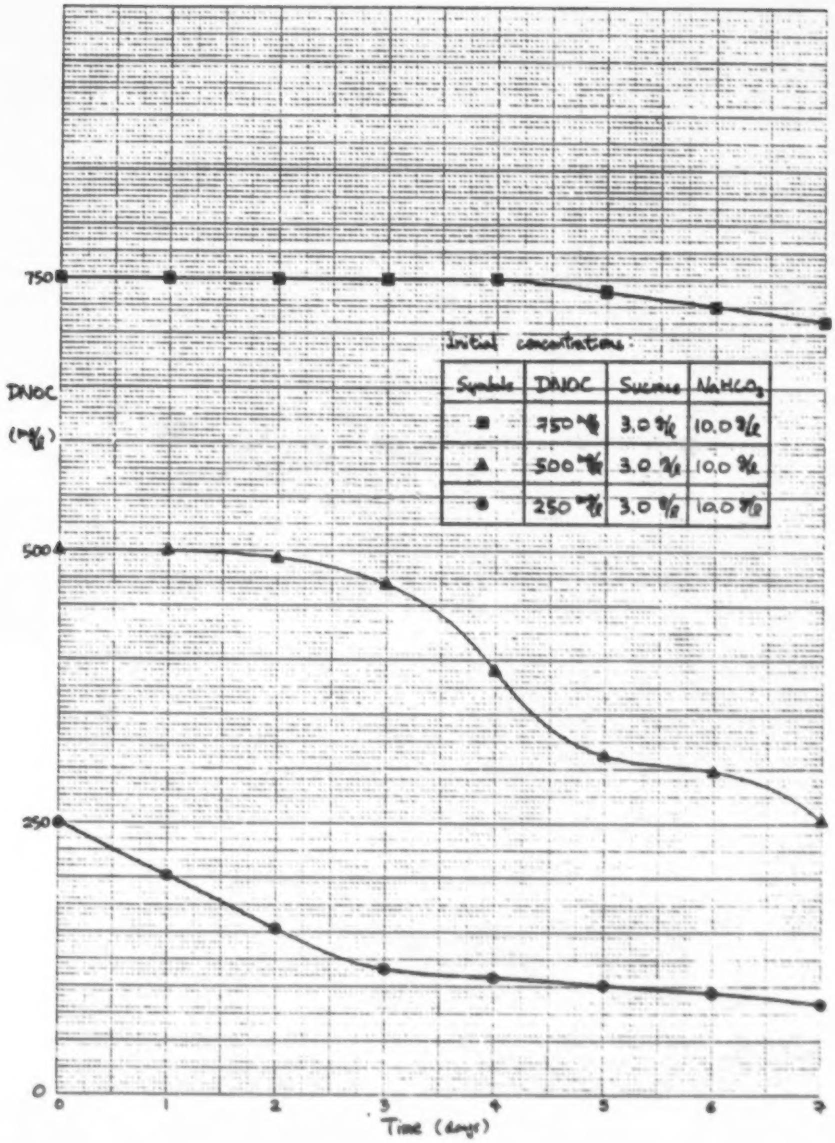

Figure B-6. DNOC changes vs. time in continuously-stirred batch test under the given initial concentrations.

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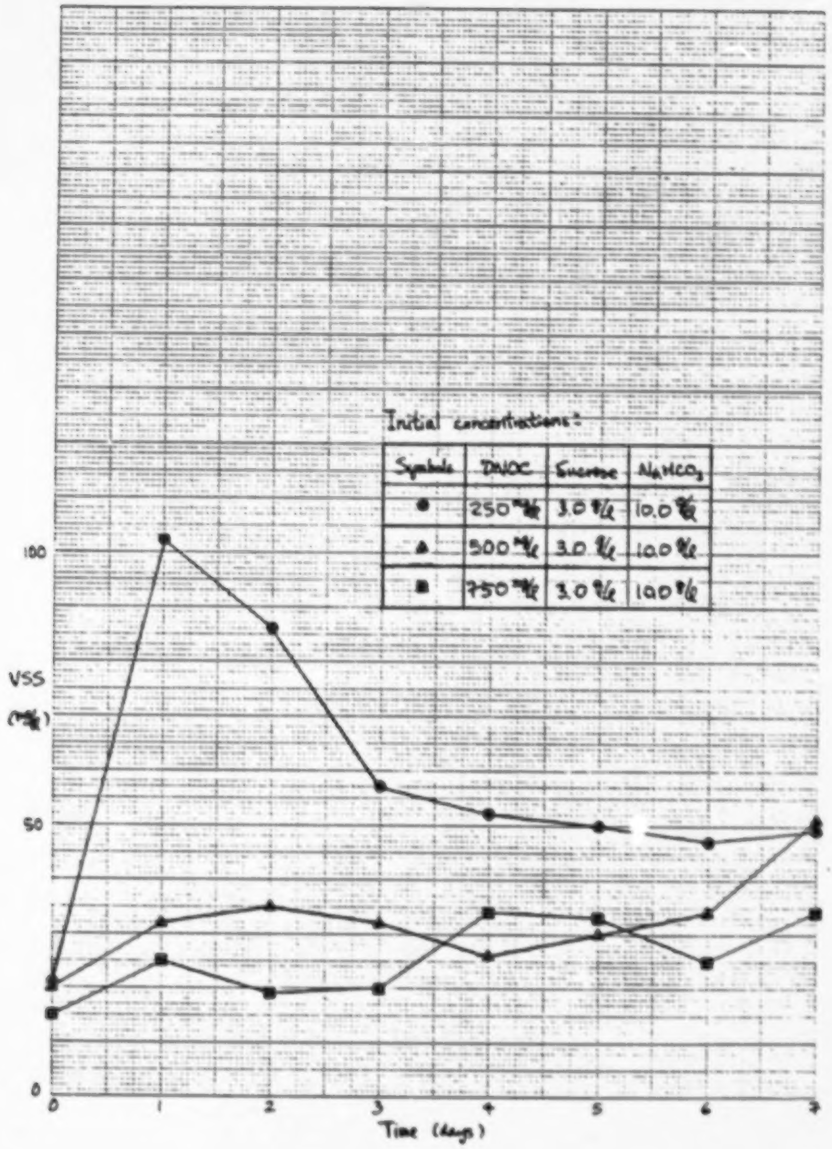

Figure B-7. VSS changes vs. time in continuously-stirred batch test under the given initial concentrations.

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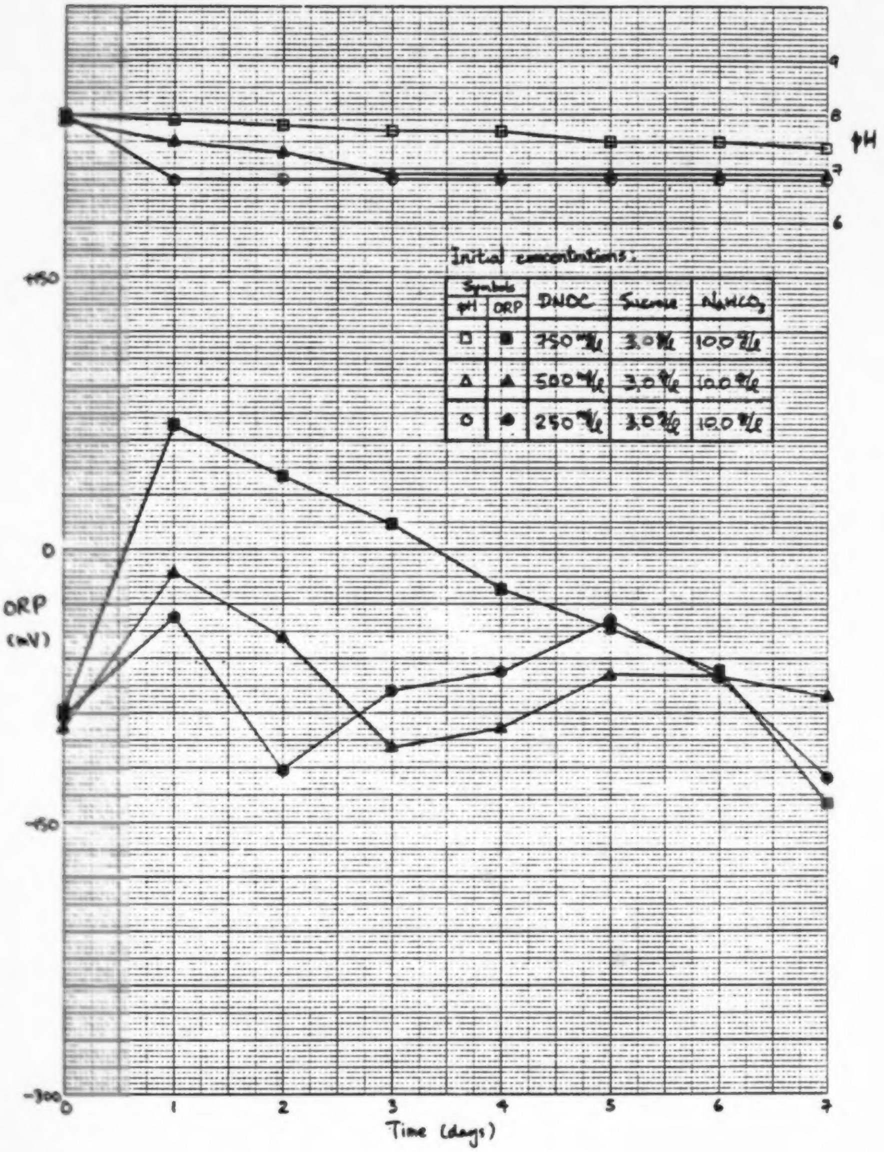

Figure B-8. pH and ORP changes vs. time in continuously-stirred batch test under the given initial concentrations.

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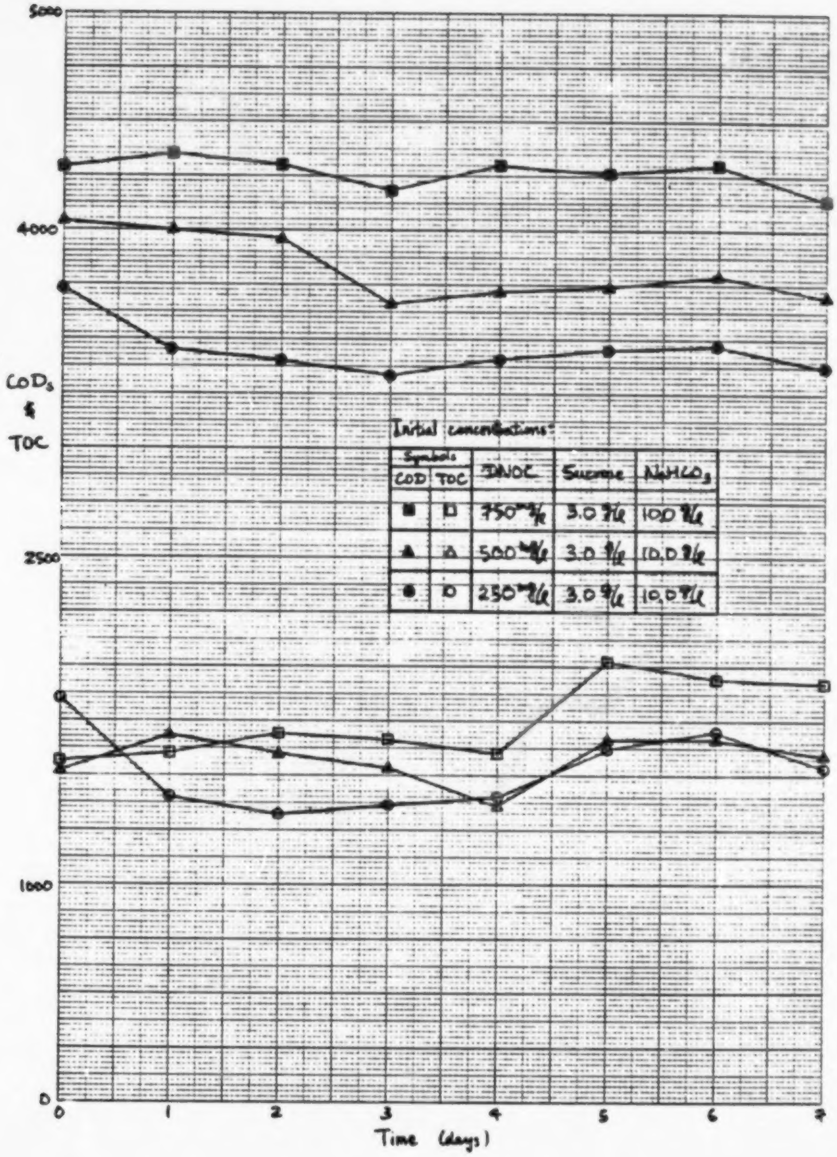
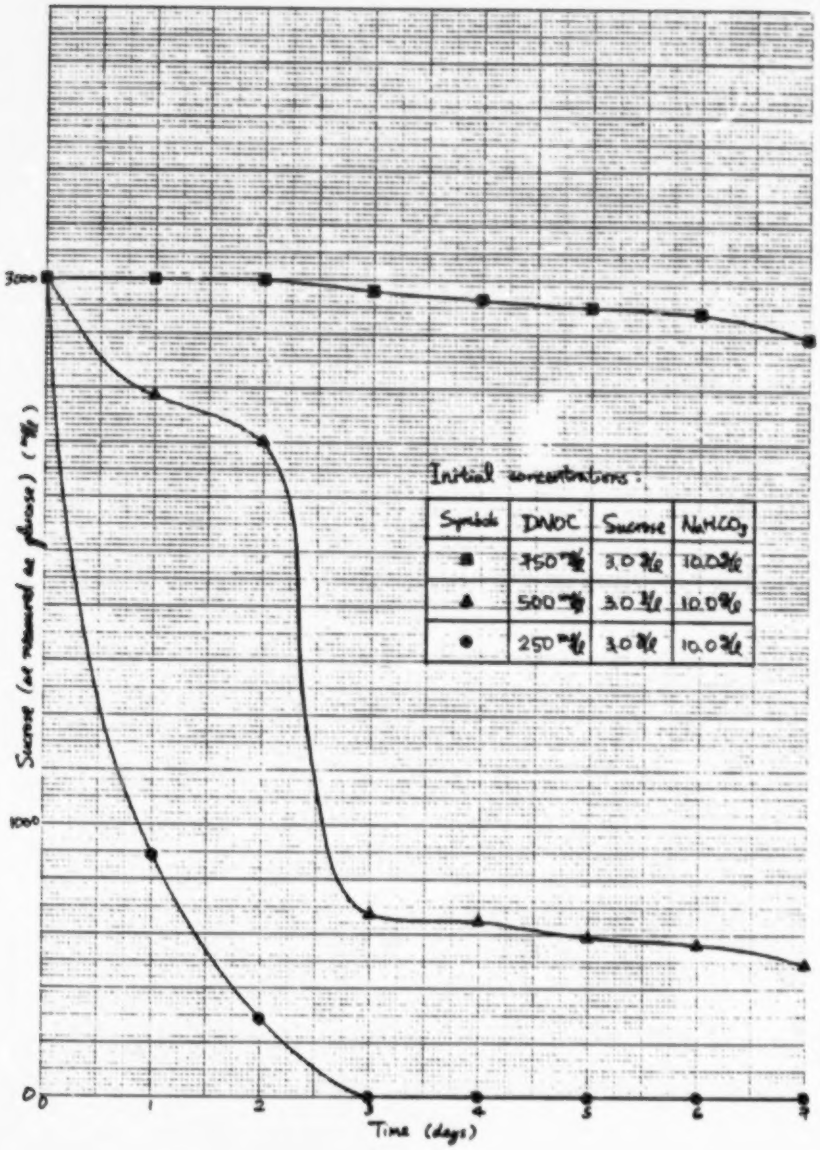


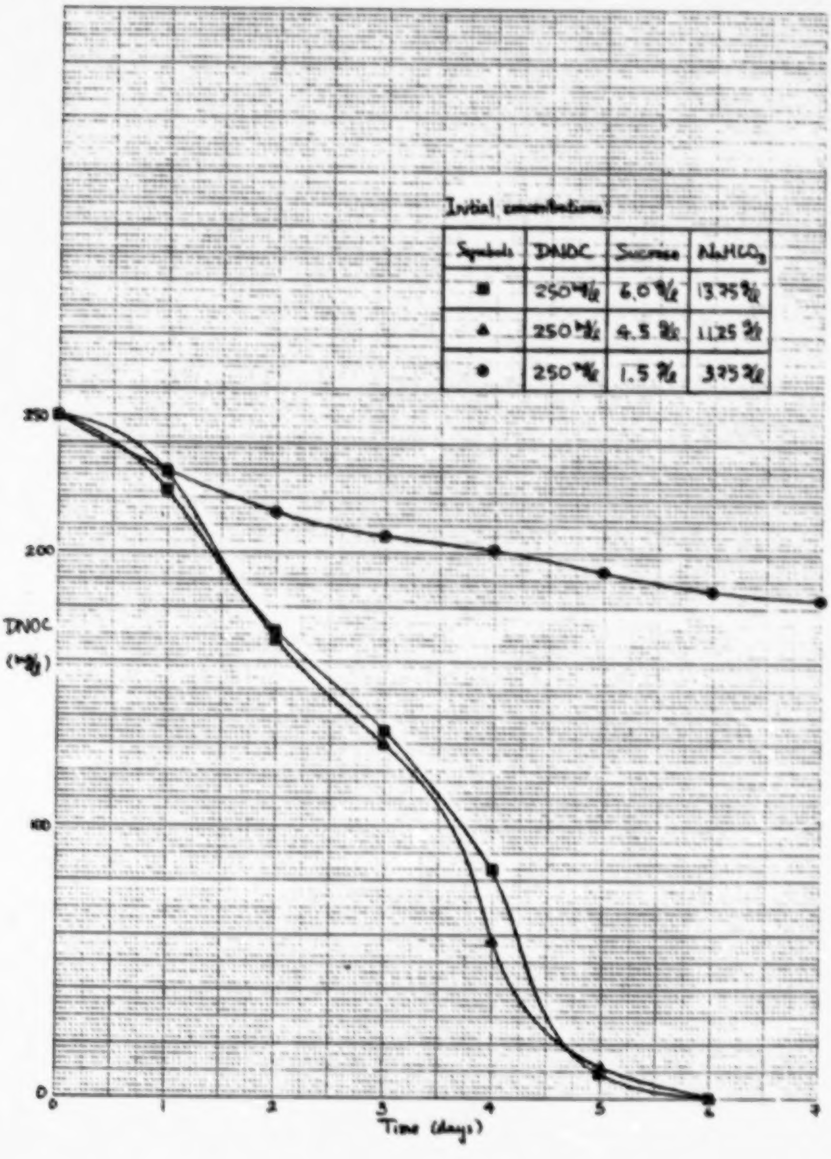
Figure B-9. COD and TOC changes vs. time in continuously-stirred batch test under the given initial concentrations.



Initial concentrations:

Symbols	DNOC	Sucrose	NaHCO$_3$
■	750 mg/l	3.0 g/l	10.0 g/l
▲	500 mg/l	3.0 g/l	10.0 g/l
●	250 mg/l	3.0 g/l	10.0 g/l

Figure B-10. Sucrose changes vs. time in continuously-stirred batch test under the given initial concentrations.



Initial concentrations

Symbols	DNOC	Sucrose	NaHCO$_3$
■	250 mg/l	6.0 g/l	13.75 g/l
▲	250 mg/l	4.5 g/l	11.25 g/l
●	250 mg/l	1.5 g/l	3.75 g/l

Figure B-11. DNOC changes vs. time in continuously-stirred batch test under the given initial concentrations.

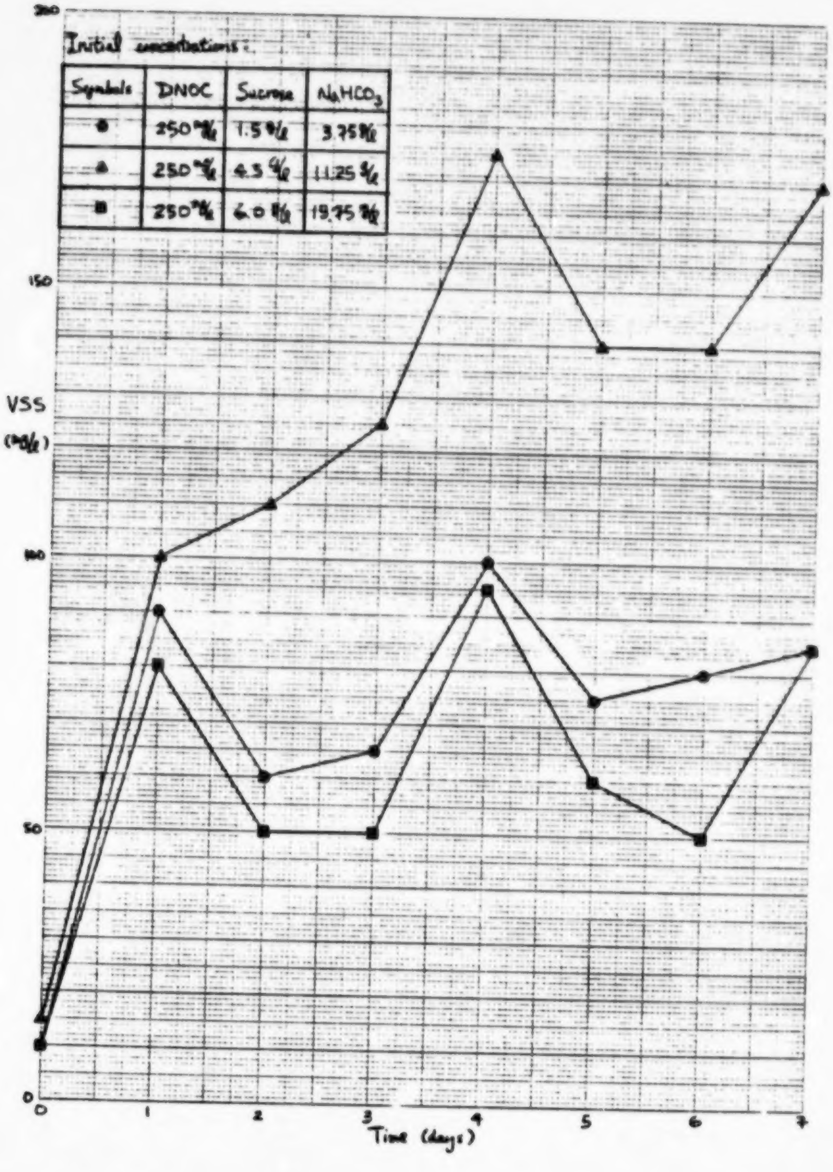


Figure B-12. VSS changes vs. time in continuously-stirred batch test under the given initial concentrations.

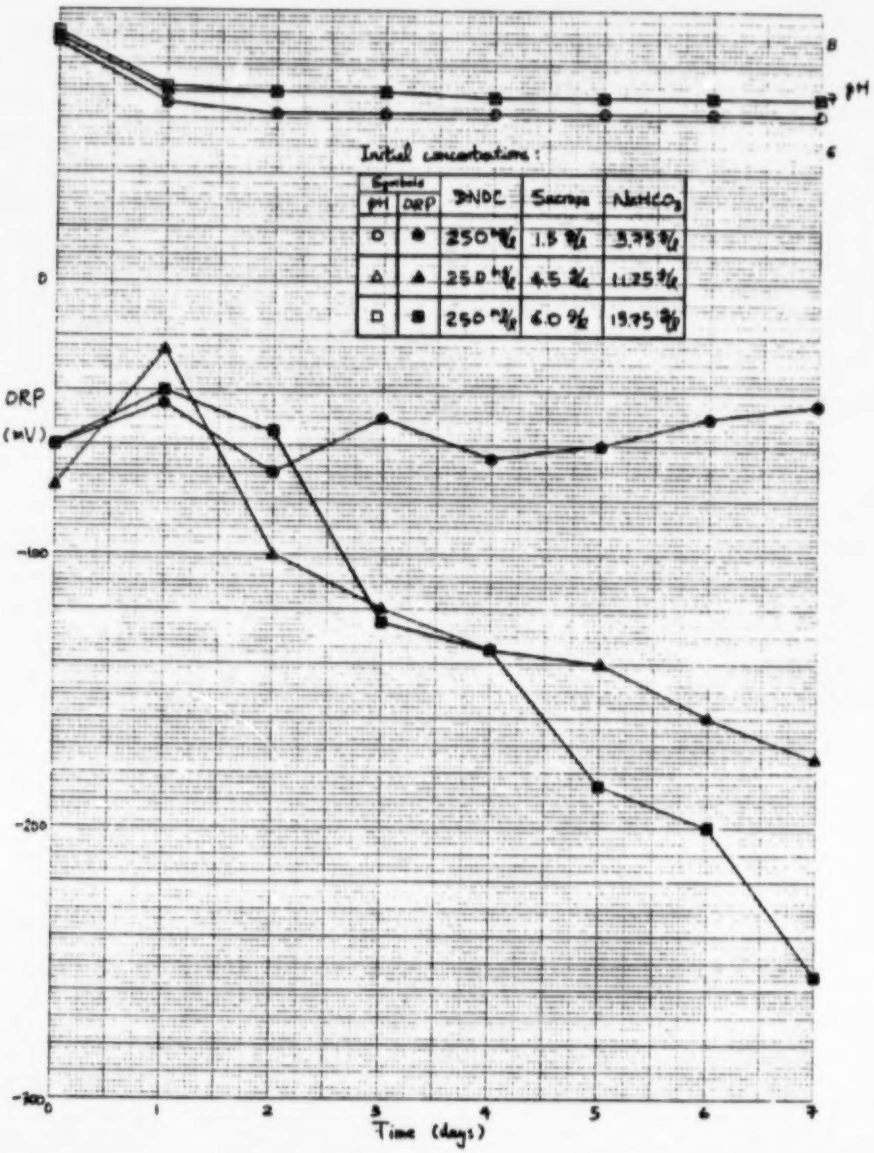


Figure B-13. pH and ORP changes vs. time in continuously-stirred batch test under the given initial concentrations.

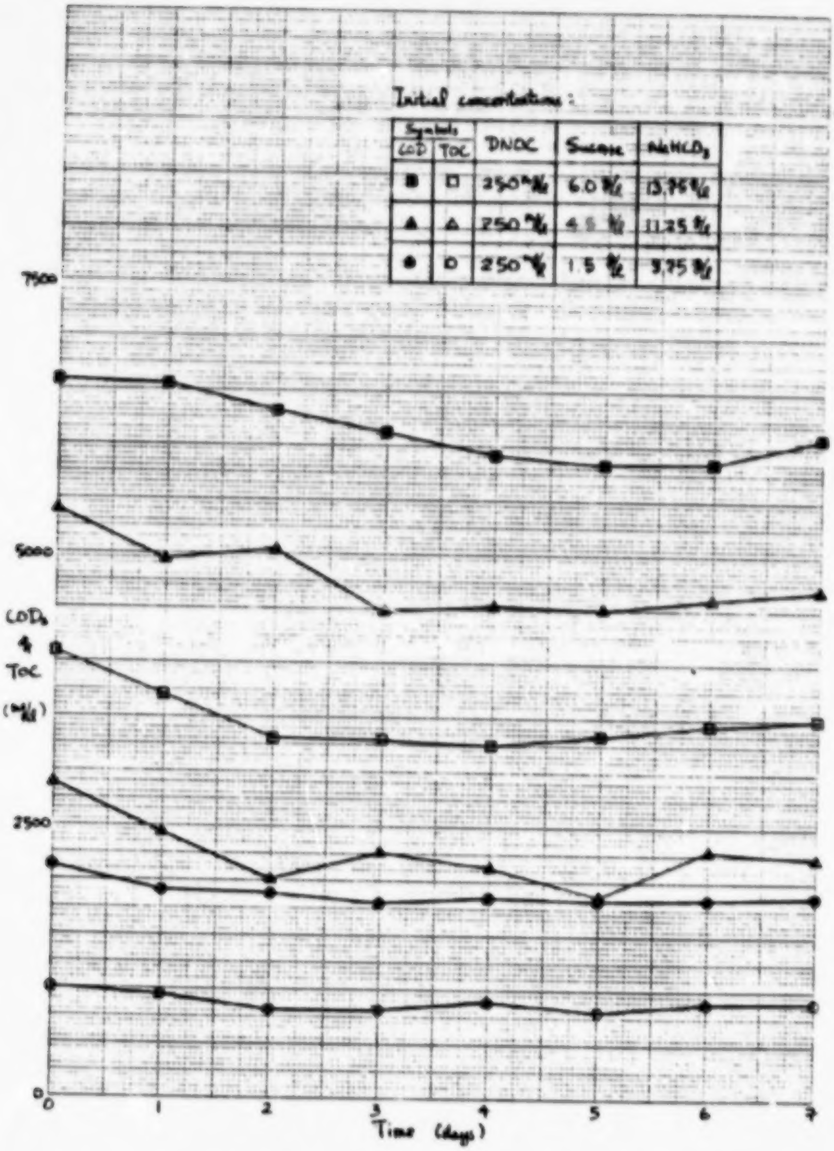


Figure B-14. COD and TOC changes vs. time in continuously-stirred batch test under the given initial concentrations.

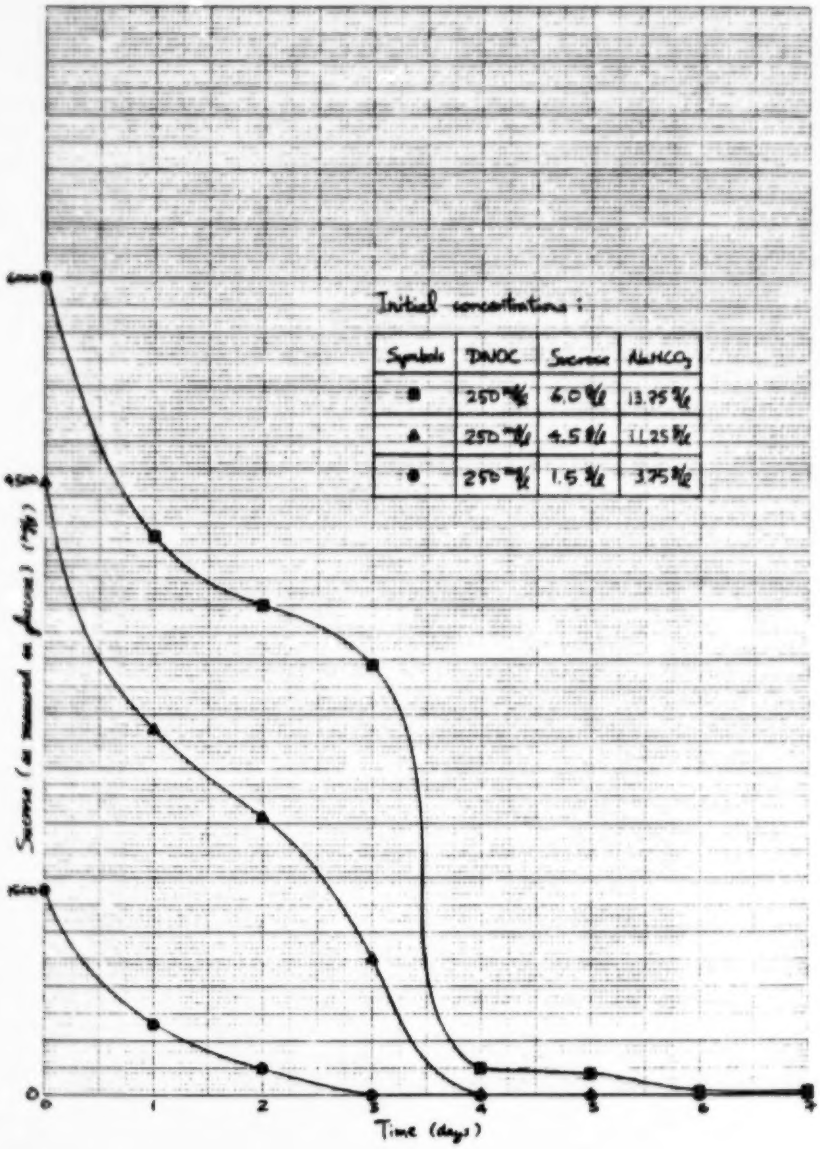


Figure B-15. Sucrose changes vs. time in continuously-stirred batch test under the given initial concentrations.

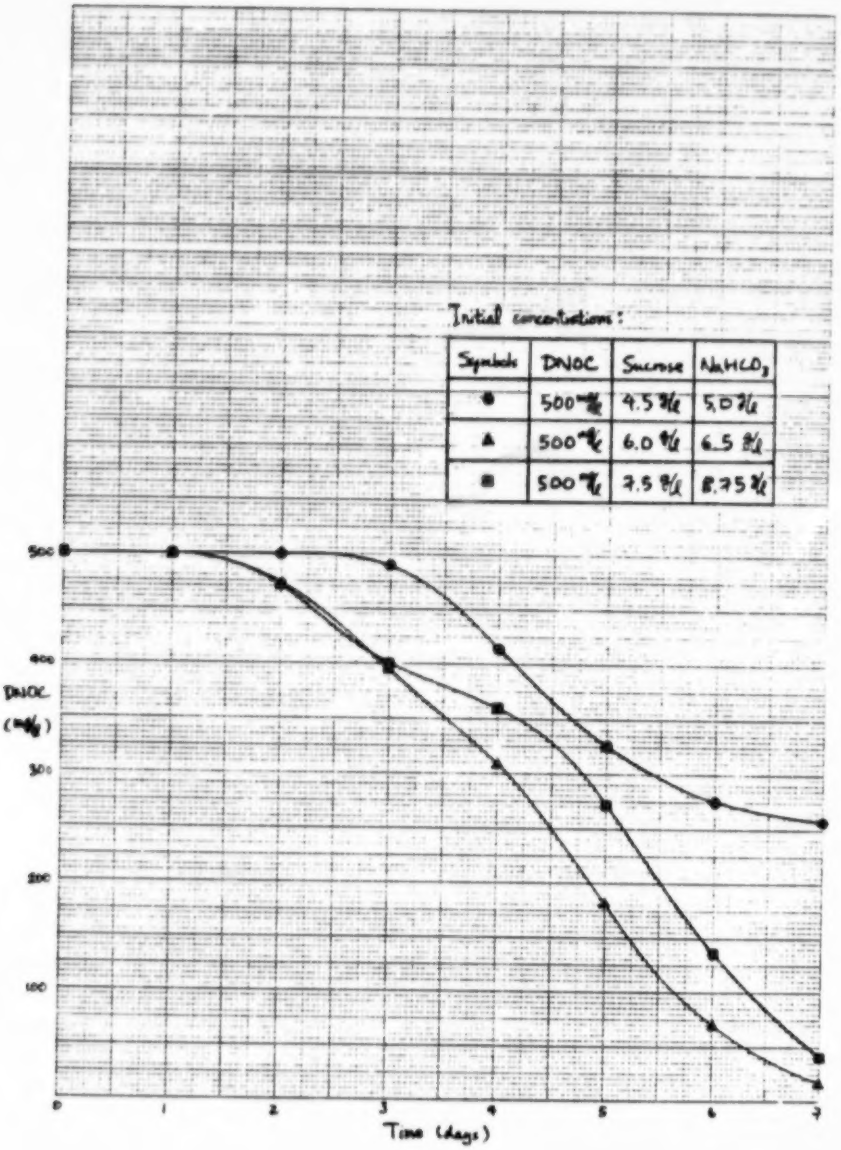


Figure B-16. DNOC changes vs. time in continuously-stirred batch test under the given initial concentrations.

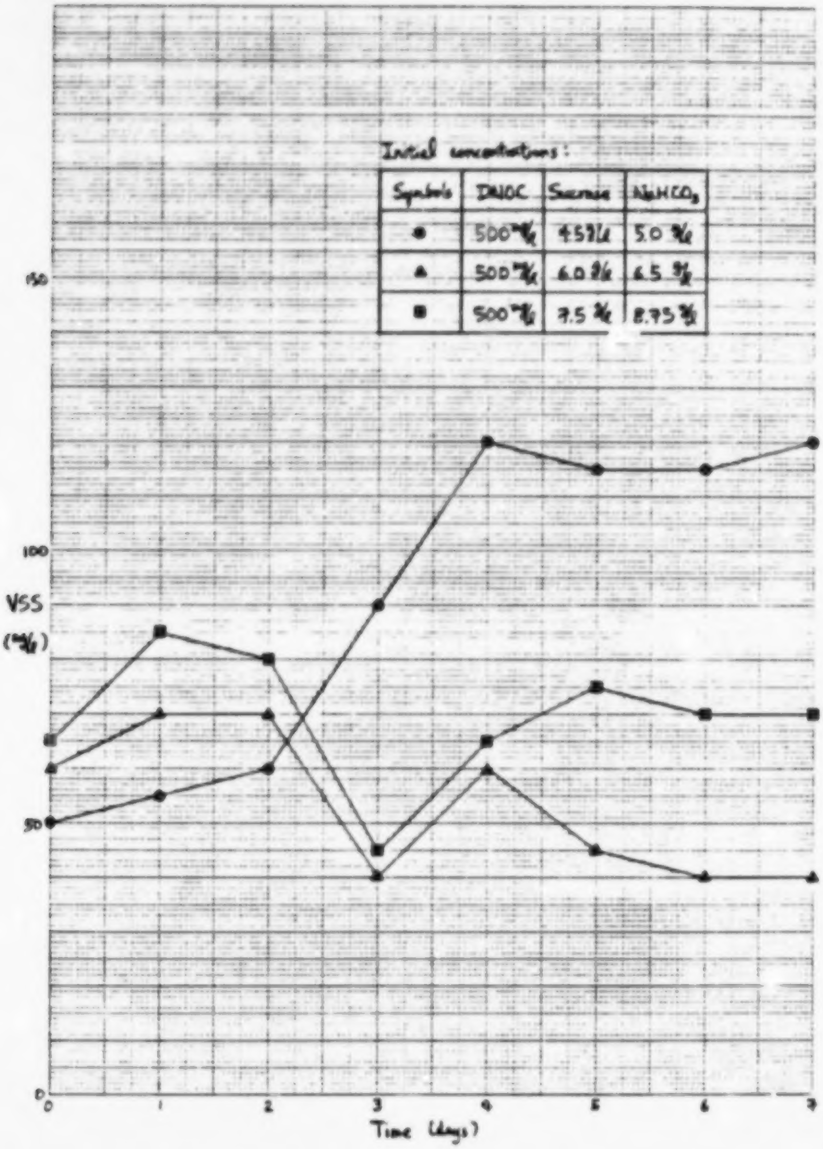


Figure B-17. VSS changes vs. time in continuously-stirred batch test under the given initial concentrations.

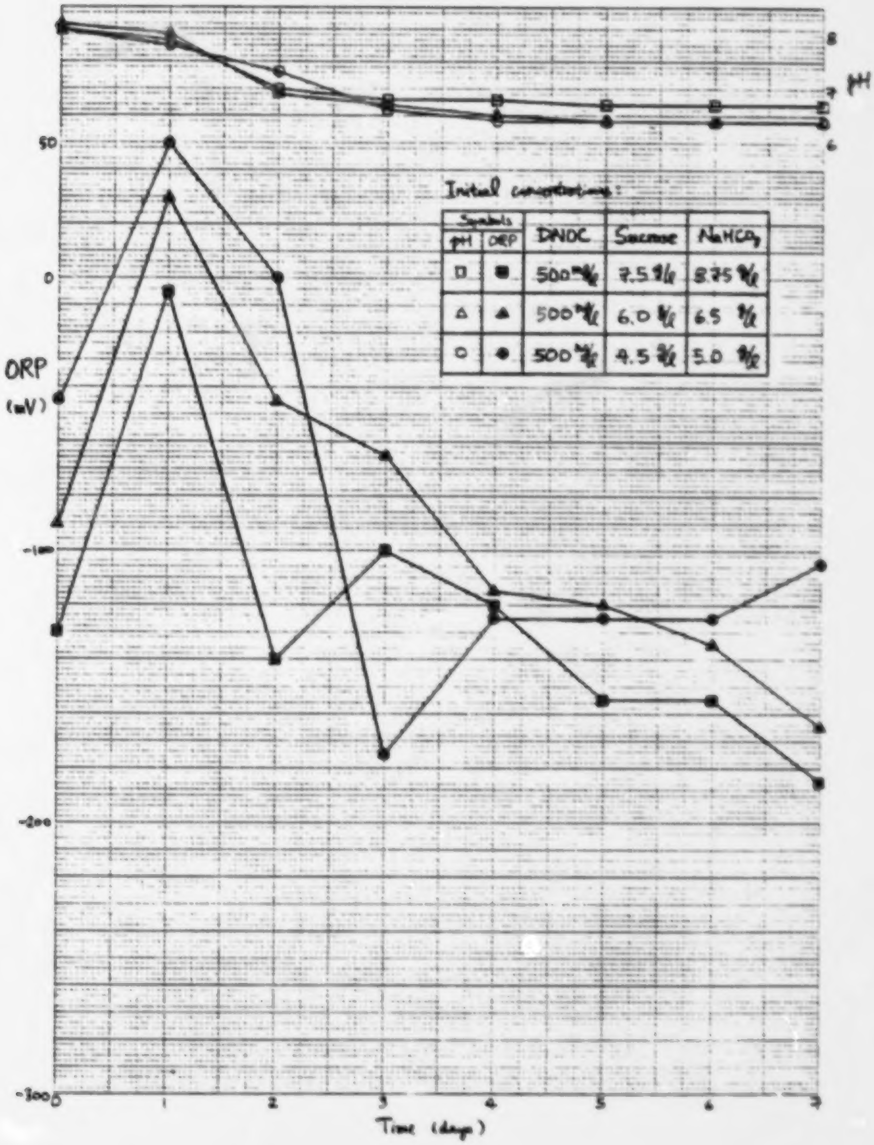


Figure B-18. pH and ORP changes vs. time in continuously-stirred batch test under the given initial concentrations.

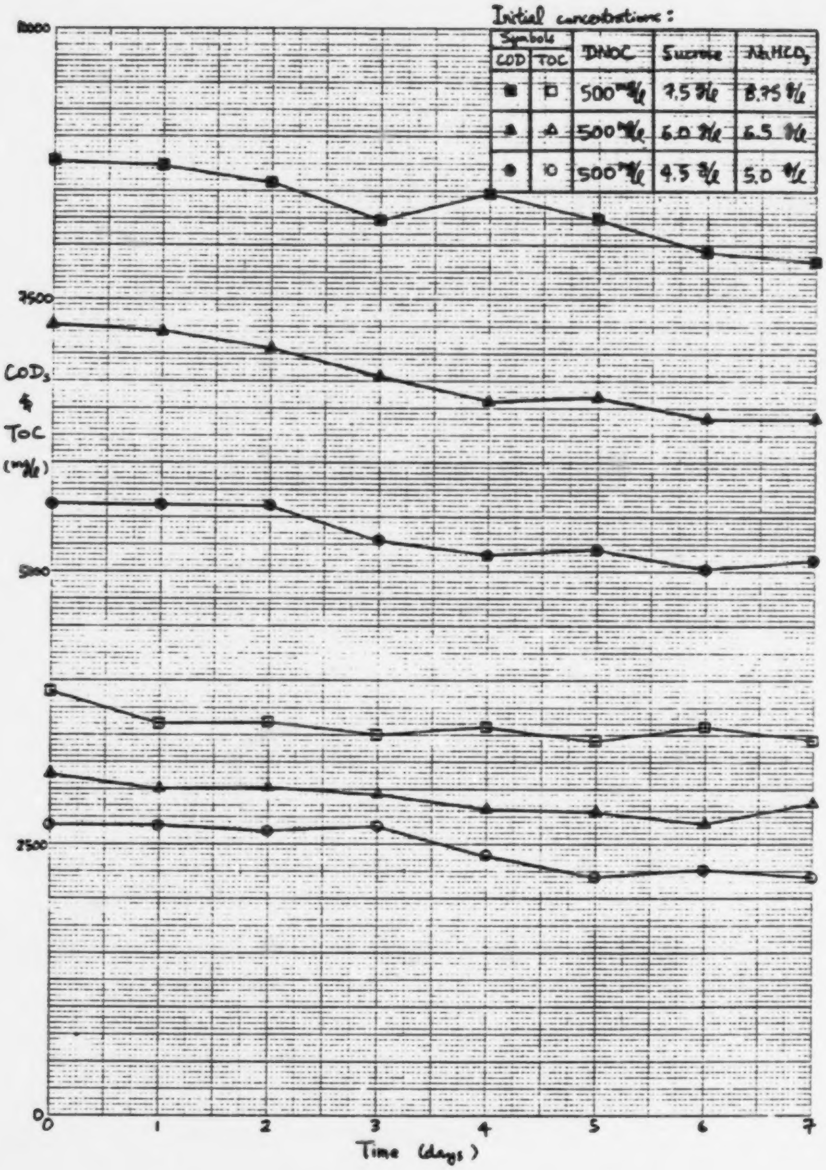


Figure B-19. COD and TOC changes vs. time in continuously-stirred batch test under the given initial concentrations.

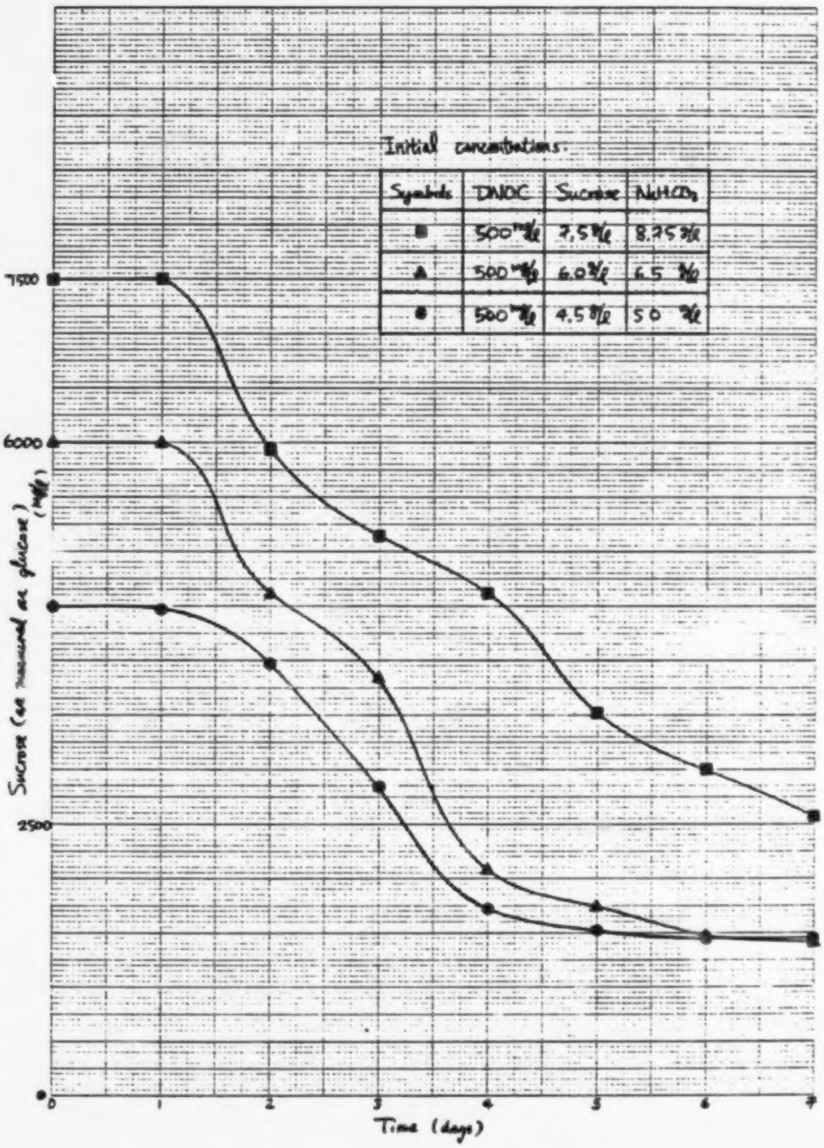


Figure B-20. Sucrose changes vs. time in continuously-stirred batch test under the given initial concentrations.

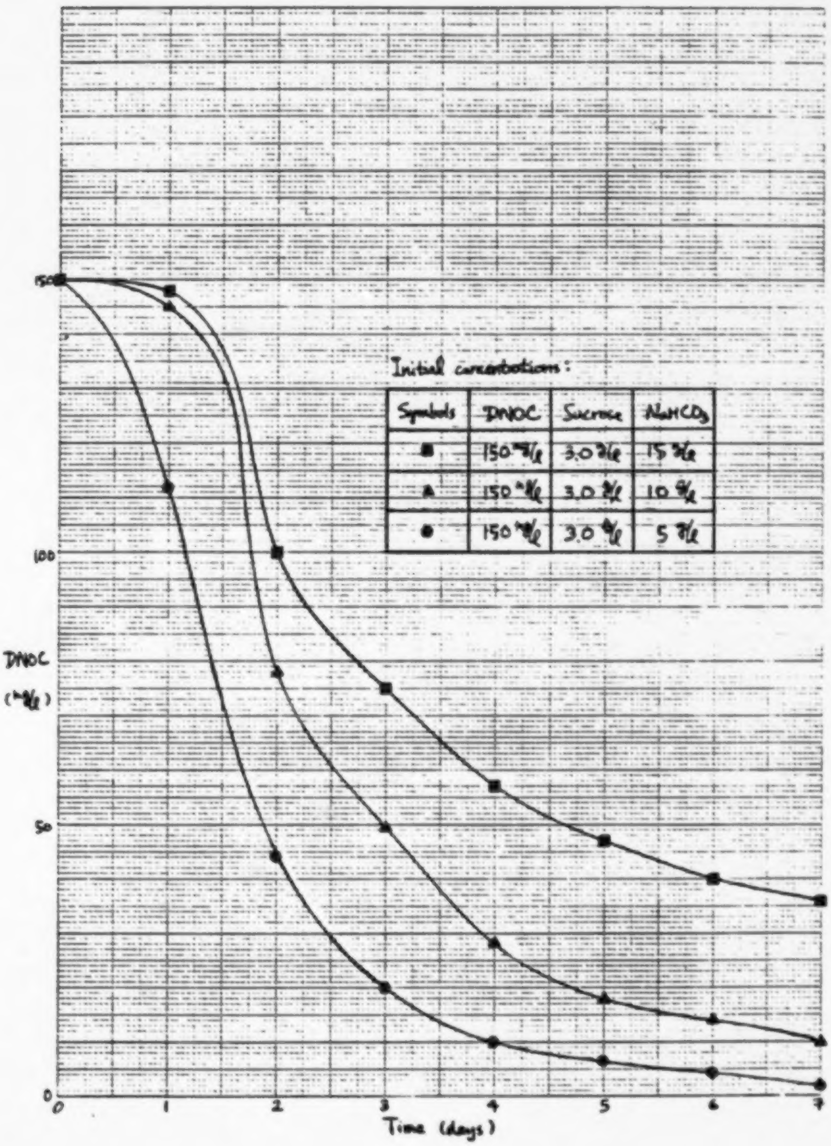


Figure B-21. DNOC changes vs. time in continuously-stirred batch test under the given initial concentrations.

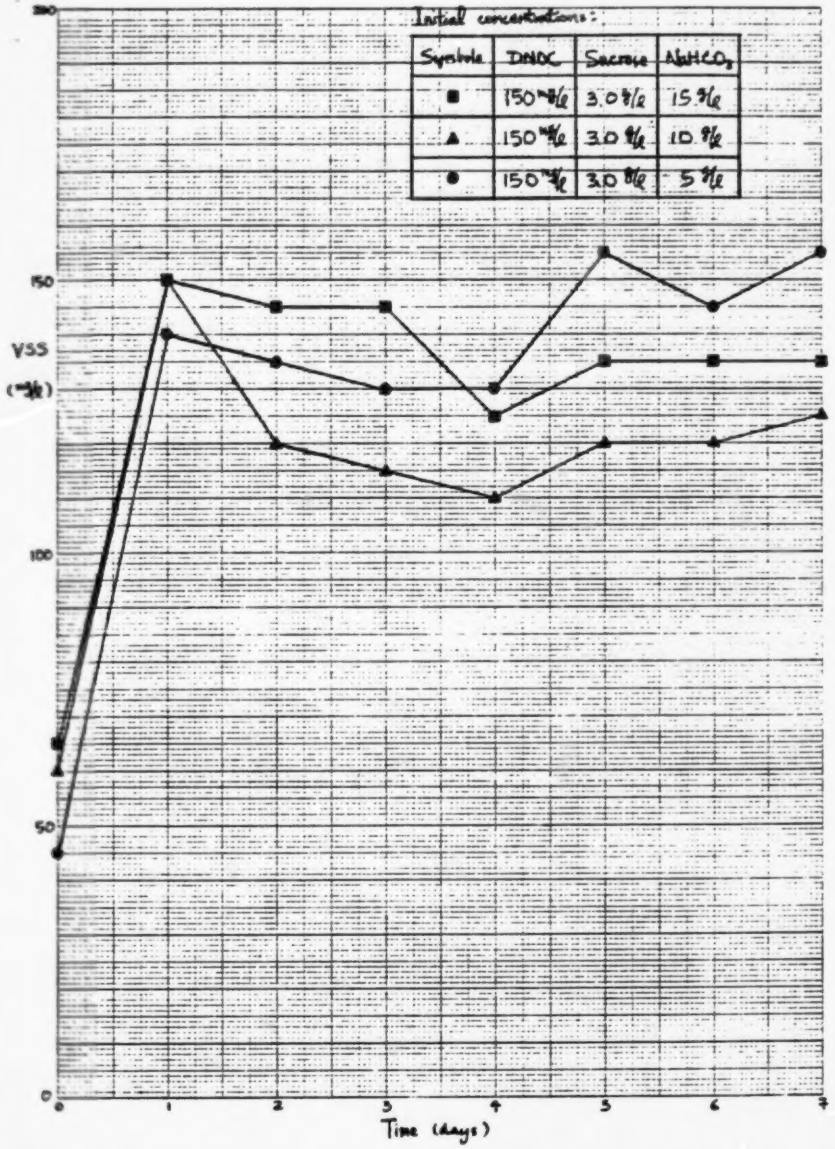


Figure B-22. VSS changes vs. time in continuously-stirred batch test under the given initial concentrations.

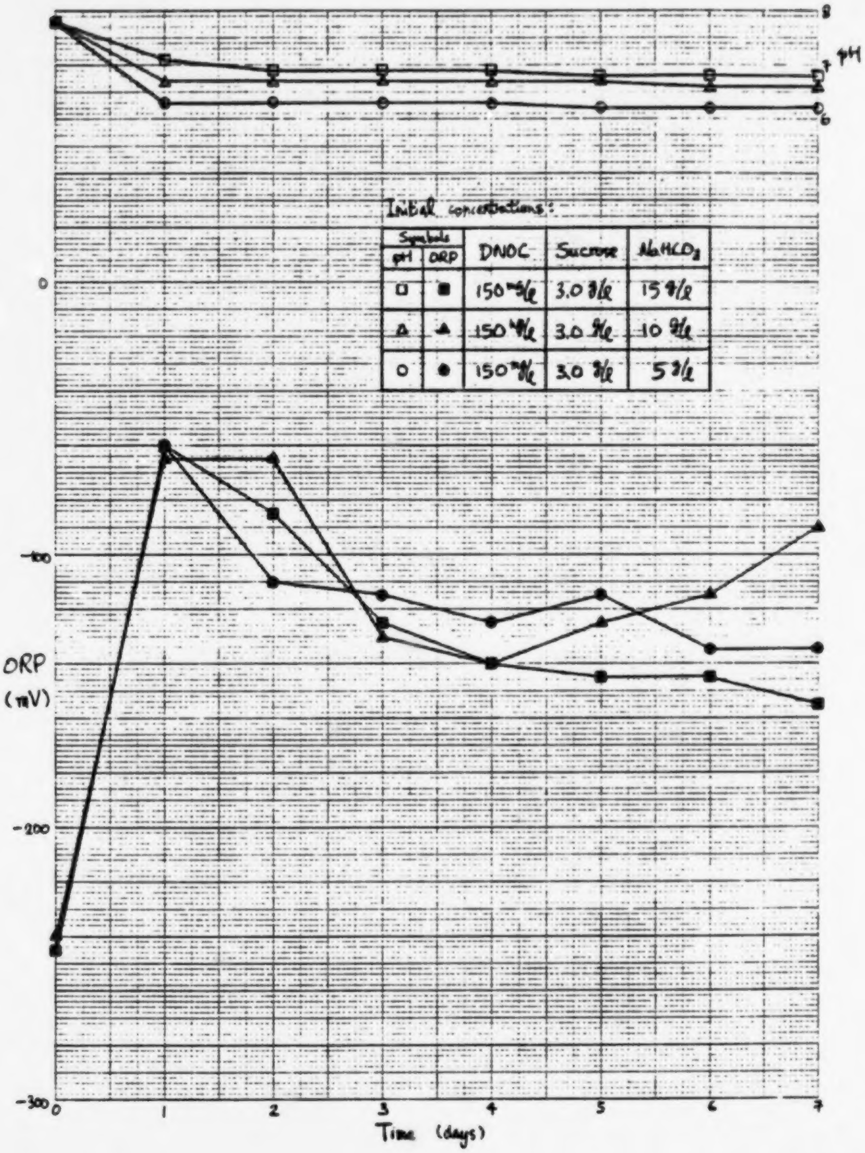


Figure B-23. pH and ORP changes vs. time in continuously-stirred batch test under the given initial concentrations.

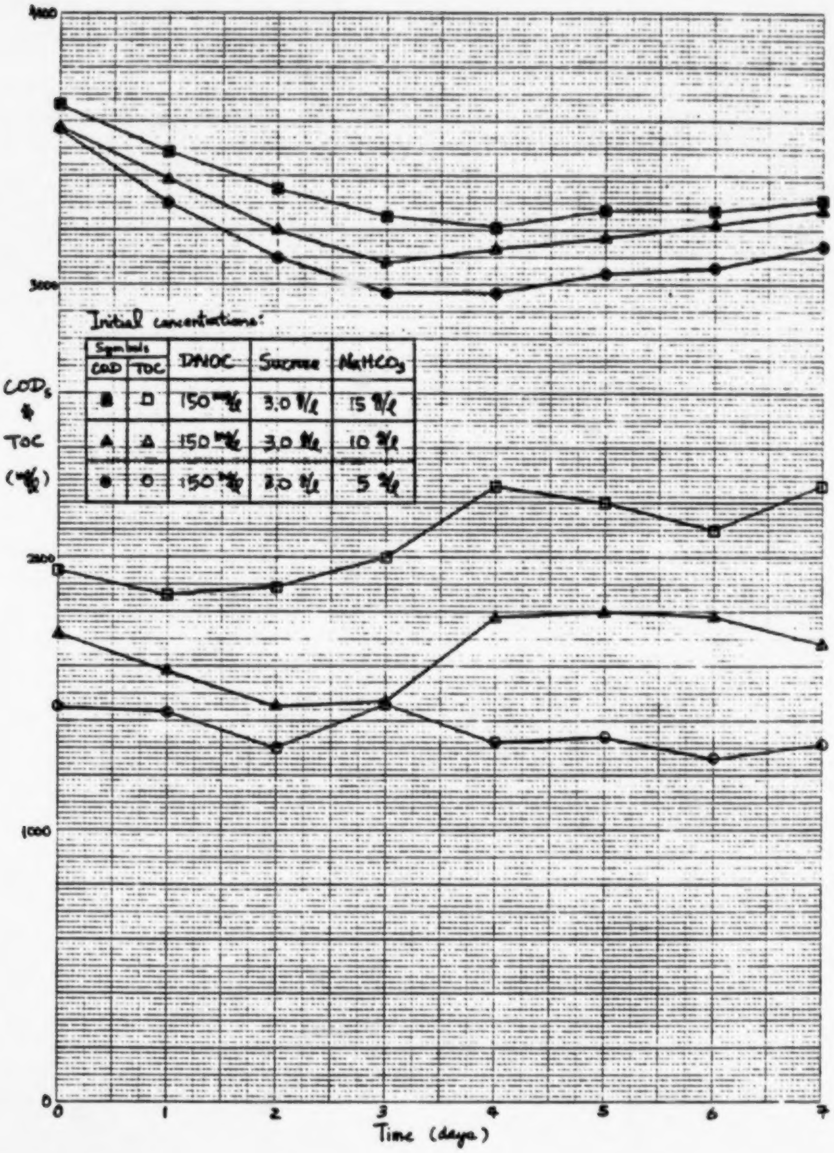


Figure B-24. COD and TOC changes vs. time in continuously-stirred batch test under the given initial concentrations.

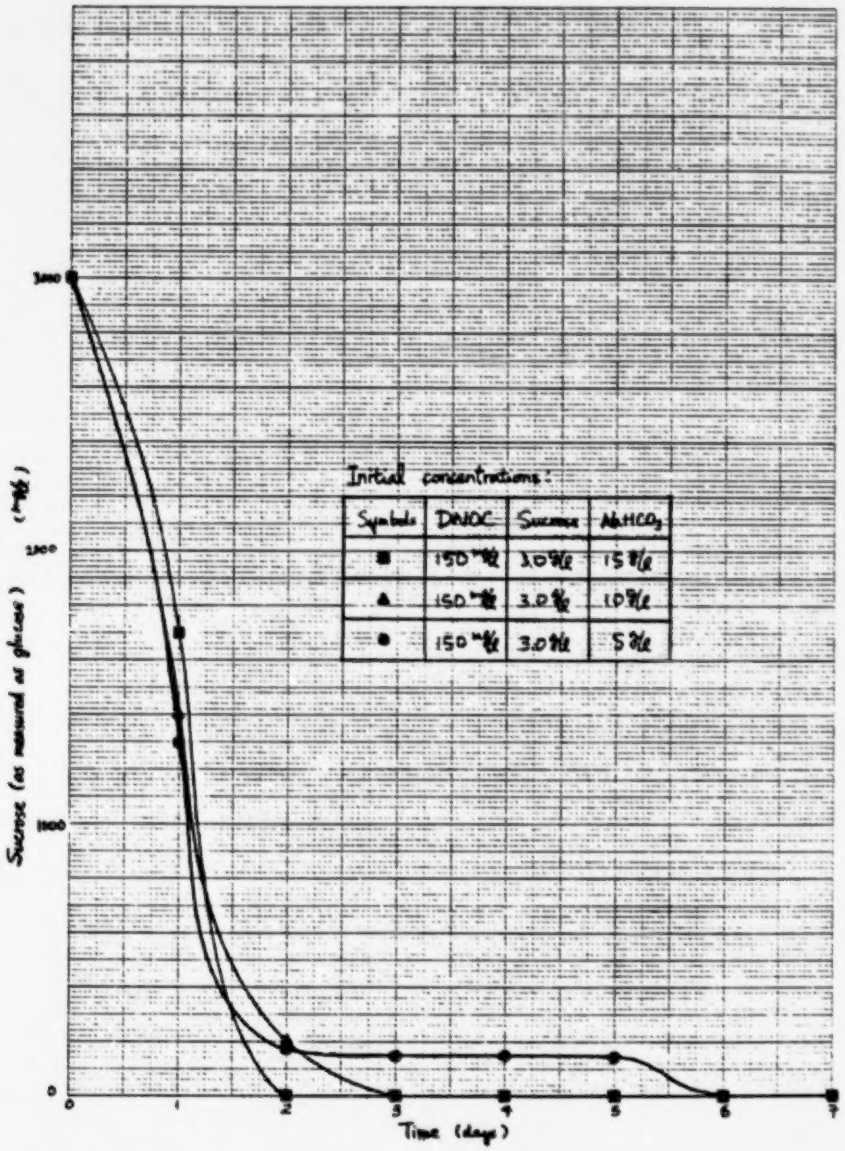


Figure B-25. Sucrose changes vs. time in continuously-stirred batch test under the given initial concentrations.

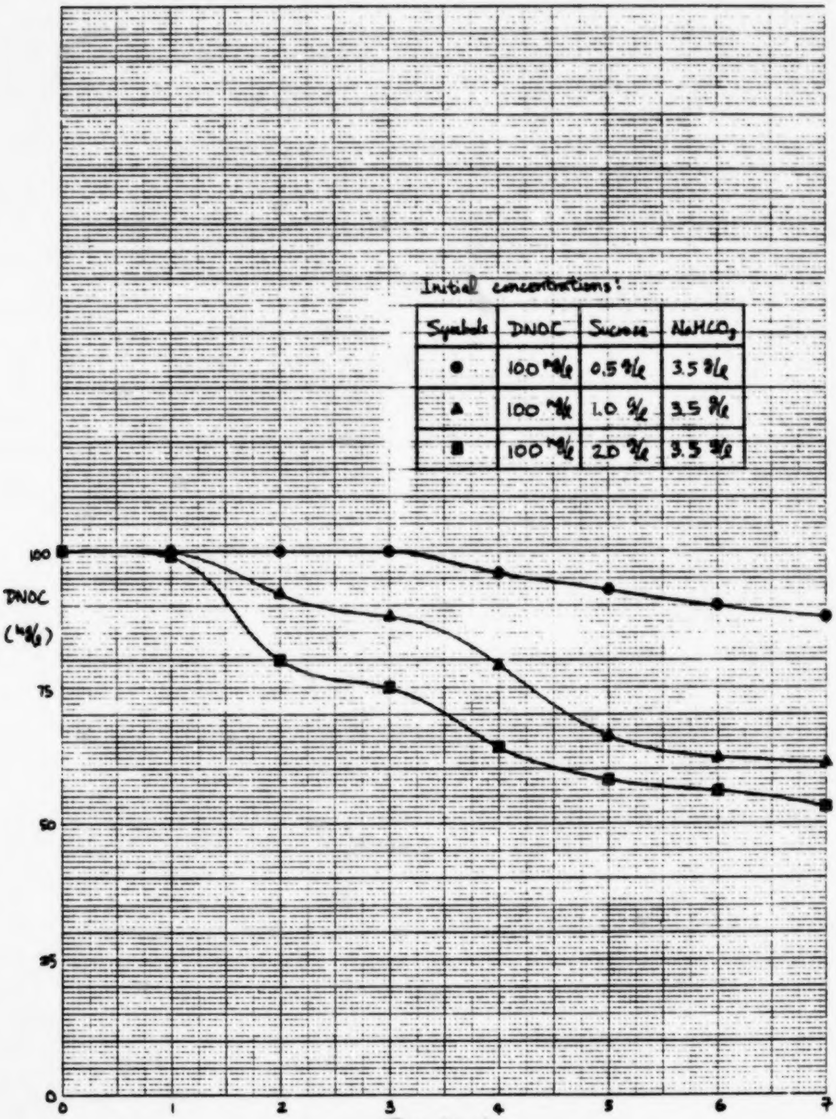


Figure B-26. DNOC changes vs. time in continuously-stirred batch test under the given initial concentrations.

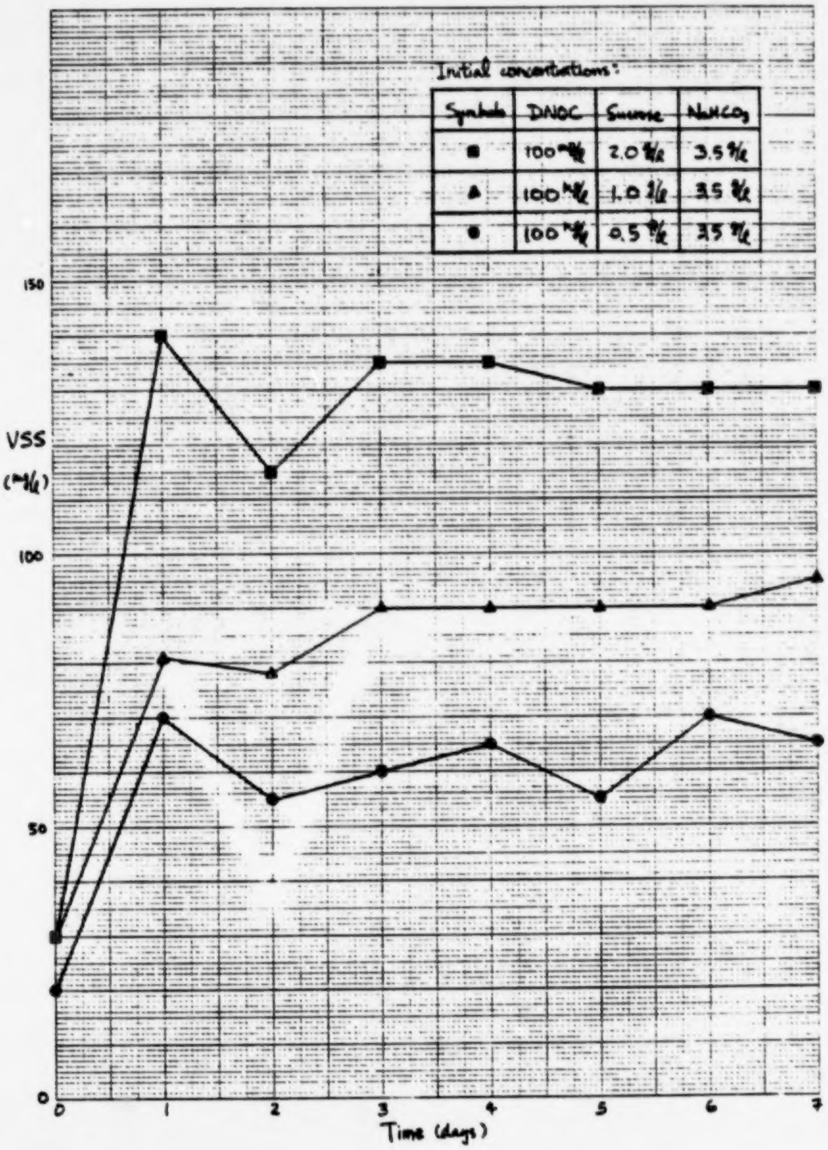


Figure B-27. VSS changes vs. time in continuously-stirred batch test under the given intial concentrations.

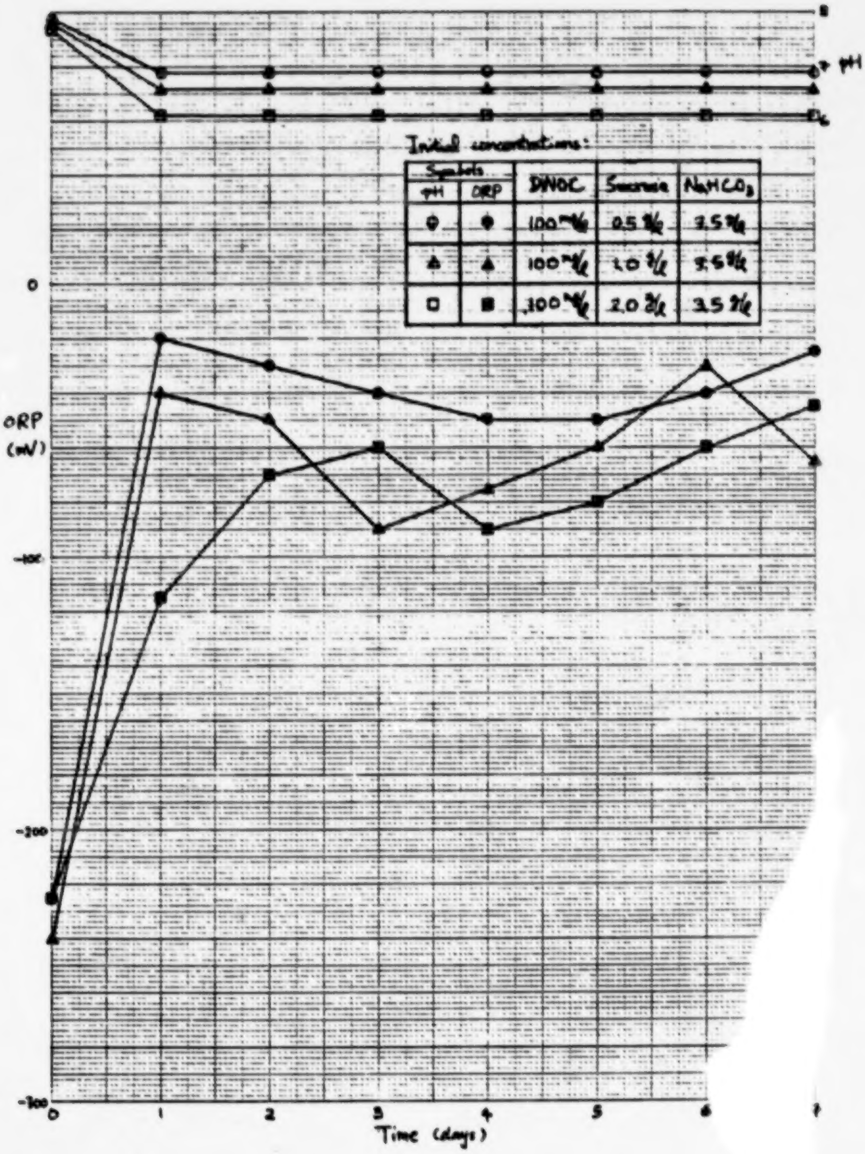


Figure B-28. pH and ORP changes vs. time in continuo -
stirred batch test under the given init
concentrations.

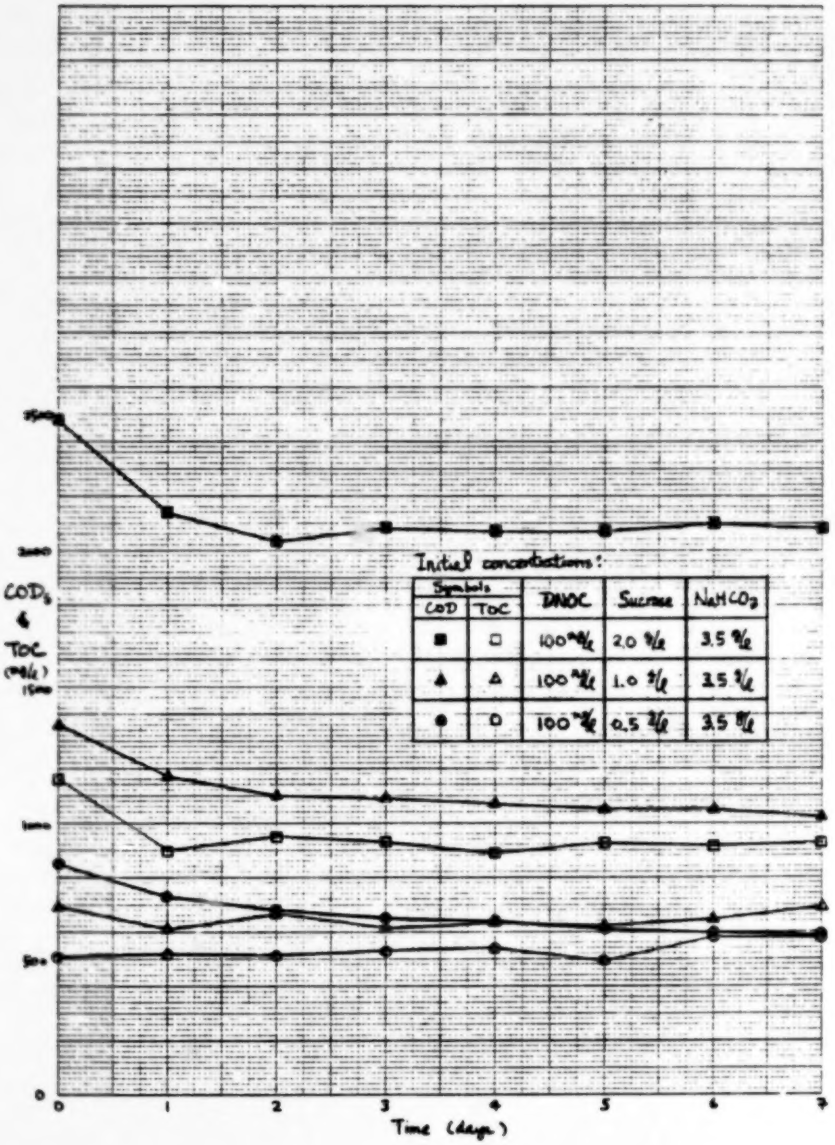


Figure B-29. COD and TOC changes vs. time in continuously-stirred batch test under the given initial concentrations.

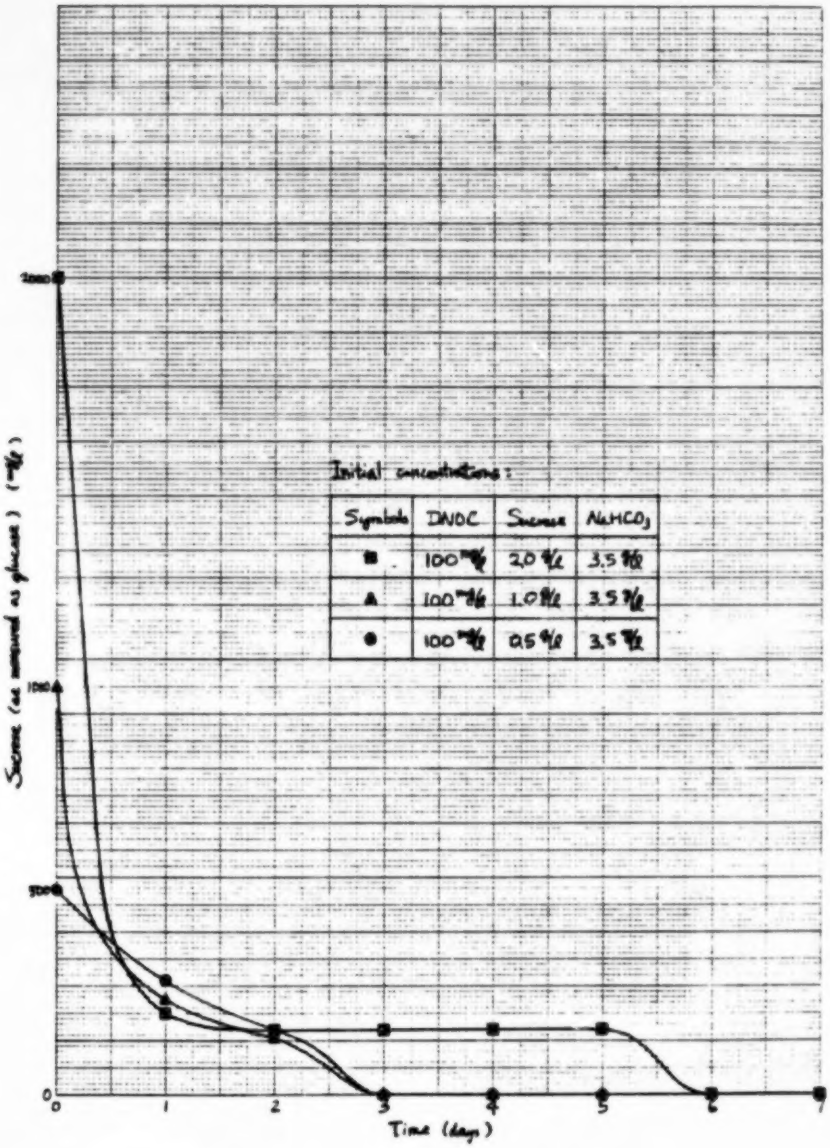


Figure B-30. Sucrose changes vs. time in continuously-stirred batch test under the given initial concentrations.

APPENDIX C

METHODS USED IN ANALYTICAL PROCEDURES

Experimental procedures employed in this study conformed to established laboratory techniques, and were in accordance with those described in "Standard Methods" (53), "Methods for Chemical Analysis of Water and Waters" (55), or "Test Methods: methods for organic chemical analysis of municipal and industrial wastewater" (56).

CHEMICAL OXYGEN DEMAND (COD) DETERMINATION

Chemical oxygen demand (COD) was measured by the modified semi-micro tube method (54,55). This method uses the same reagents as listed in Standard Methods (53), but uses smaller volumes. It has been approved by the EPA for NPDES compliance monitoring.

DNOC DETERMINATION BY GAS CHROMATOGRAPH

Equipment

Varian Aerograph 1440 gas chromatograph with flame ionization detector.

Column: 3 ft x 2 mm ID Glass. Packing: 1% SP-1240 DA on 100/120 supelcoport. Supplier: Supelco, Inc.

Carrier gas: Prepurified N_2 at 20 ml/min and 24 psig.

Flame ionization detector fuel: prepurified H_2 (29 ml/min, 15 psig) and air (300 ml/min, 30 psig).

Temperatures: 180°C injector and 180°C detector.

Column temperature program: 2 min initial hold at 150°C, 15°C/min to 170°C and hold.

Procedure

The procedures for sample preparation, extraction, quality control and data interpretation followed Method 604-Test Method for Phenols (56).

OFF-GAS DETERMINATION

Volumetric measurements of off-gas produced in the anaerobic column were done following volumetric method-511A of Standard Methods (53).

Off-gas composition was determined following gas chromatographic method-511B of Standard Method (53), using 1 l sealed gas sampling bags for sample collection.

Equipment

Baseline Industries, Inc. Model 1030A gas chromatograph equipped with a thermal conductivity detector. Helium was used as carrier gas, analytical grade CO_2, CH_4 and N_2 were used as calibration gases.

OXIDATION-REDUCTION POTENTIAL (ORP) DETERMINATION

The determination of oxidation-reduction potential (ORP) was done by the use of Sensorex reference combination ORP electrode (Sensorex Inst. Co., Westminister, California) along with a Fisher Accumet^R 325 pH/Ion Meter (Fisher Scientific Co., Pittsburg, Pennsylvania).

pH DETERMINATION

pH was measured with a Fisher Accumet^R 325 pH/Ion Meter (Fisher Scientific Co., Pittsburg, Pennsylvania).

SUCROSE DETERMINATION

The determination of sucrose concentration was done by using the indirect method in which sucrose is inverted by hydrolysis to give equal parts of glucose and fructose. Samples and sucrose standard curve samples were hydrolyzed with H_2SO_4 and the concentration of glucose formed after the inversion was determined by Beckman Glucose Analyzer II.

SUSPENDED SOLIDS (SS) AND VOLATILE SUSPENDED SOLIDS (VSS) DETERMINATIONS

Suspended solids (SS) were determined using Method 209D of Standard Method (53) for total nonfiltrable residue dried at 103-105°C, and volatile suspended solids (VSS) were determined using Method 209G for volatile and fixed matter in nonfiltrable residue and in solid and semisolid samples.

TOTAL ORGANIC ACIDS (TOA) DETERMINATION

Total organic acids (TOA) were measured by the chromatographic separation method-504A of Standard Methods (53). However, when a sample contains a high concentration of DNOC or its by-products, the identification of the final titration endpoint is not clear due to interference by the yellowish or reddish color of DNOC or its anaerobic degradation products.

TOTAL ORGANIC CARBON (TOC) DETERMINATION

Total organic carbon (TOC) analyses were made in a Beckman Carbonaceous Analyzer Model 915B equipped with total and inorganic carbon channels.

TOXICITY DETERMINATION

Toxicity measurements were done by using the Beckman Microtox^{TM} toxicity analyzer. The bioassay method used is based on the light output of luminescent bacteria, as measured by a temperature controlled photometric device. The bioassay microorganisms are handled like a chemical reagent.

Toxicity analysis procedures as described in Microtox^{TM} system operating manual (51) were followed.

The EC_{50}, which is the effective concentration that reduces the light output by 50%, was determined by plotting gamma (the ratio of the amount of light lost to the amount of light remaining) as a function of sample concentration on log-log graph paper. The EC_{50} value was determined by the intersection of the best fit line with gamma = 1.0.